PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 39/395, C07K 16/42

A1

(11) International Publication Number: **WO 97/33616**

(43) International Publication Date: 18 September 1997 (18.09.97)

(21) International Application Number: PCT/US97/03443

(22) International Filing Date: 6 March 1997 (06.03.97)

(30) Priority Data:
08/614,220 12 March 1996 (12.03.96) US

(71) Applicants: GENENTECH, INC. [US/US]; 460 Point San Bruno Boulevard, South San Francisco, CA 94080-4990 (US). JOHNS HOPKINS UNIVERSITY [US/US]; 720 Rutland Avenue, Baltimore, MD 21205 (US).

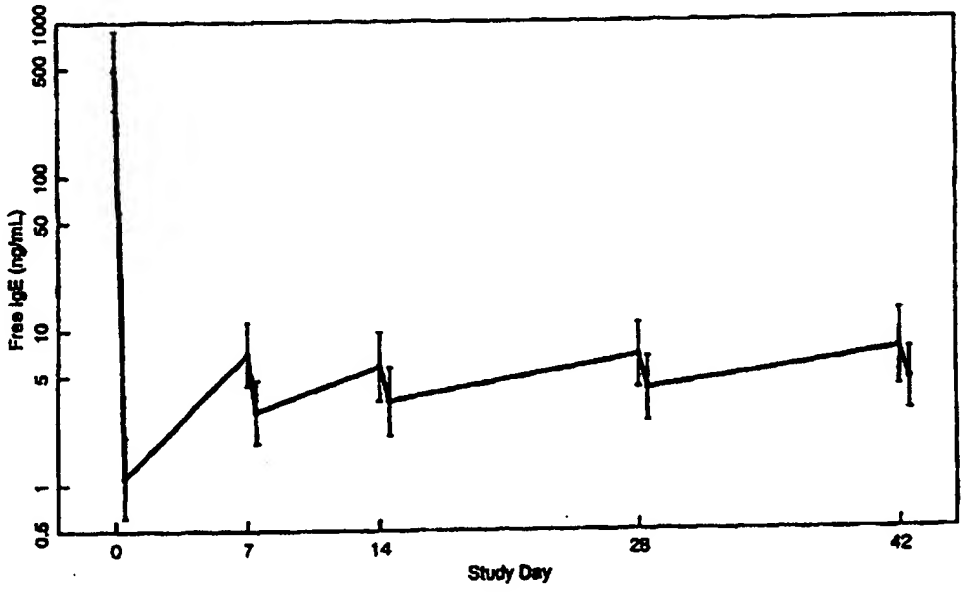
(72) Inventors: MACGLASHAN, Donald, W., Jr.; 221 Rickswood Road, Timonium, MD 21093 (US). ADELMAN, Daniel, C.; 23 Woodleaf Avenue, Redwood City, CA 94061 (US). REIMANN, James, D.; 2315 Jefferson #5, Berkeley, CA 94703 (US).

(74) Agents: SVOBODA, Craig, G. et al.; Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, CA 94080-4990 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHODS FOR TREATMENT OF ALLERGIC DISEASES WITH IGE



(57) Abstract

Methods of treatment of allergic diseases, e.g. allergic rhinitis and allergic asthma, with anti-IgE antibodies and other IgE antagonists are described.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgystan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic			SE	Sweden
CG	Congo	KR	Republic of Korea	SG	Singapore
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LR	Liberia	SZ	Swaziland
CS	Czechoslovakia	LT	Lithuania	TD	Chad
CZ	Czech Republic	LU	Luxembourg	TG	Togo
DE	Germany	LV	Latvia	TJ	Tajikistan
DK	Denmark	MC	Monaco	TT	Trinidad and Tobago
EE	Estonia	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	UG	Uganda
FI	Finland	ML	Mali	US	United States of America
FR	France	MN	Mongolia	UZ	Uzbekistan
GA	Gabon	MR	Mauritania	VN	Viet Nam

METHODS FOR TREATMENT OF ALLERGIC DISEASES WITH IGE

This invention relates to methods of treatment of allergic diseases with IgE antagonists, including anti-IgE antibodies.

BACKGROUND OF THE INVENTION

Allergic rhinitis is the most common form of atopic disease, affecting approximately 25%-30% of the U.S. population. Ragweed is the most common cause of seasonal allergic rhinitis (hay fever) in North America and is widespread throughout the warmer portions of the Western Hemisphere.

Current therapies for the treatment of allergic rhinitis symptoms include allergen avoidance, pharmacological interventions (e.g. antihistamines, sympathomimetics, topical and systemic corticosteroids and chromones) and immunotherapy. Although helpful, many of these pharmacological interventions provide only moderate or partial relief of symptoms and may be associated with significant side effects. Traditional allergy immunotherapy can be employed alone or in conjunction with these pharmacological interventions to provide relief of symptoms, but is only effective in a narrow, antigen-specific fashion.

Asthma is a common condition characterized by reversible airflow obstruction and airway hyperresponsiveness, attributed to bronchial inflammation. It is not understood precisely how airway hyperreactivity is produced in predisposed individuals but allergen-induced inflammatory responses appear to be pivotal in many forms of clinical asthma. In this regard, allergen exposure can increase airway responsiveness in sensitized subjects and, in the allergic subject with asthma, can lead to worsening and persistence of asthma symptoms.

IgE plays a central role in the cascade of events leading to the early and late airway responses to allergen exposure in allergic asthma. The interaction of allergen with mast cell bound IgE triggers aggregation and crosslinking of IgE receptors, in turn provoking mast cell degranulation with the release of preformed mediators, such as histamine and tryptase, and the synthesis and release of prostaglandins, leukotrienes, and cytokines. These inflammatory mediators are thought to cause the airway mucosal edema and contraction of smooth muscle responsible for the immediate bronchoconstriction that characterizes the early asthmatic response to allergen challenge.

Airway hyperreactivity reactions can be induced in the laboratory by exposing subjects with allergic asthma to nebulized solutions of allergen extract, the concentration of which can be determined by airway hyperreactivity to methacholine and skin test reactivity to the same allergen. This procedure is known as experimental aerosolized allergen challenge or bronchial provocation. Bronchial provocation is a useful and relevant model for the study of anti-asthma medications (Cockcroft *et al. J. Allergy Clin. Immunol.* 79:734-40 (1987); Cresciolli *et al. Ann. Allergy* 66:245-51 (1991); Ward *et al. Am. Rev. Respir. Dis.* 147:518-23 (1993)). For example, it is known that beta agonists inhibit the early asthmatic response (EAR) but not the late asthmatic response (LAR) to allergen, and that a single dose of inhaled steroid inhibits the LAR but not the EAR (Cockcroft *et al., J. Allergy Clin. Immunol.* 79:734-40 (1987)). Theophylline and disodium cromoglycate attenuate both the EAR and LAR responses to allergen (Cresciolli *et al., supra;* Ward *et al.*

supra). Most drugs with proven efficacy in asthma management have been shown to attenuate airway responses to inhaled antigens administered in bronchial provocation.

Other allergic diseases, such as allergic rhinitis, result when IgE antibodies bind to FcεRI receptors located on circulating basophils. Like the functioning of mast cells in allergic asthma, basophils mediate allergic disease by release of histamine and other mediators upon allergen crosslinking of allergen-specific IgE antibodies occupying FcεRI receptors on the basophil cell surface. Malveaux *et al., J. Clin. Invest.*, **62**: 176-181 (1978) reported a study of human basophil receptor density and noted an excellent correlation between the plasma total IgE level and the number of FcεRI receptors per basophil. This and subsequent studies noted that FcεRI densities in allergic and non-allergic individuals range from 10^4 to 10^6 receptors per basophil.

Allergic rhinitis symptoms can be induced by exposing subjects to nasal challenge with allergen. There is a highly significant correlation between the results of nasal challenge and nasal symptoms among rhinitic patients (Bousquet *et al., J. Aller. Clin. Immunol.*, **85**: 490-497 (1990)). In addition, many drugs with proven efficacy in rhinitis management have been shown to attenuate symptoms induced by nasal challenge with allergen. Nasal challenge is a useful model for demonstrating the efficacy of anti-allergy medications (see e.g., Naclerio et *al., Arch. Otolaryngol.*, **110**: 25-27 (1984); Bascom *et al., J. Aller. Clin. Immunol.*, **81**: 580-589 (1988)).

The concept of using anti-IgE antibodies as a treatment for allergy has been widely disclosed in the scientific literature. A few representative examples are as follows. Baniyash and Eshhar (*European Journal of Immunology* **14**:799-807 (1984)) demonstrated that an anti-IgE monoclonal antibody could specifically block passive cutaneous anaphylaxis reaction when injected intradermally before challenging with the antigen; U.S. 4,714,759 discloses a product and process for treating allergy, using an antibody specific for IgE; and Rup and Kahn (*International Archives Allergy and Applied Immunology*, **89**:387-393 (1989) discuss the prevention of the development of allergic responses with monoclonal antibodies which block mast cell-IgE sensitization.

Anti-IgE antibodies that block the binding of IgE to its receptor on basophils yet fail to bind to IgE bound to the receptor, thereby avoiding histamine release are disclosed, for example, by Rup and Kahn (supra), by Baniyash *et al. (Molecular Immunology* **25**:705-711, 1988), and by Hook *et al. (Federation of American Societies for Experimental Biology*, 71st Annual Meeting, Abstract #6008, 1987).

Antagonists of IgE in the form of receptors, anti-IgE antibodies, binding factors, or fragments thereof have been disclosed in the art. For example, U.S. 4,962,035 discloses DNA encoding the alpha-subunit of the mast cell IgE receptor or an IgE binding fragment thereof. Hook *et al.* (*Federation Proceedings* Vol. 40, No. 3, Abstract #4177) disclose monoclonal antibodies, of which one type is anti-idiotypic, a second type binds to common IgE determinants, and a third type is directed towards determinants hidden when IgE is on the basophil surface.

U.S. 4,940,782 discloses monoclonal antibodies which react with free IgE and thereby inhibit IgE binding to mast cells, and react with IgE when it is bound to the B-cell membrane, but do not bind with IgE when it is bound to the mast cell Fcε receptor, nor block the binding of IgE to the B-cell receptor.

U.S. 4,946,788 discloses a purified IgE binding factor and fragments thereof, and monoclonal antibodies which react with IgE binding factor and lymphocyte cellular receptors for IgE, and derivatives thereof.

U.S. 5,091,313 discloses antigenic epitopes associated with the extracellular segment of the domain which anchors immunoglobulinsto the B cell membrane. The epitopes recognized are present on IgE-bearing B cells but not basophils or in the secreted, soluble form of IgE. U.S. 5,252,467 discloses a method for producing antibodies specific for such antigenic epitopes. U.S. 5,231,026 discloses DNA encoding murine-human antibodies specific for such antigenic epitopes.

U.S. 4,714,759 discloses an immunotoxin in the form of an antibody or an antibody fragment coupled to a toxin to treat allergy.

Presta *et al. (J. Immunol.* **151**:2623-2632 (1993)) disclose a humanized anti-IgE antibody that prevents the binding of free IgE to FcεRI but does not bind to FcεRI-bound IgE. Copending WO 93/04173 discloses polypeptides which bind differentially to the high- and low-affinity IgE receptors.

U.S. 5,428,133 discloses anti-IgE antibodies as a therapy for allergy, especially antibodies which bind to IgE on B cells, but not IgE on basophils. This publication mentions the possibility of treating asthma with such antibodies. U.S. 5,422,258 discloses a method for making such antibodies.

Tepper et al. ("The Role of Mast cells and IgE in Murine Asthma", presented at "Asthma Theory to Treatment", July 15-17, 1995) disclose that neither mast cells nor IgE greatly influence the anaphylaxis, airway hyperreactivity, or airway inflammation in a murine model of asthma.

SUMMARY OF THE INVENTION

The invention provides for a method for treating an allergic disease, including allergic rhinitis and allergic asthma, comprising administering a loading dose of an IgE antagonist for a period of at least about 14 days sufficient to reduce the patient's average serum free IgE level to a level no greater than about 50 ng/ml at the end of the loading period, followed by a maintenance dose of the IgE antagonist averaging about 0.00008 to 0.0024 mg/kg/week IgE antagonist for every IU/ml baseline free IgE in the patient's serum, wherein the maintenance dose is at least about three fold lower than the loading dose in units of mg/kg/week IgE antagonist for every IU/ml baseline free IgE in the patient's serum.

The invention further provides for a method for treating allergic disease, including allergic rhinitis and allergic asthma, comprising administering a loading dose of an IgE antagonist averaging at least about 0.003 mg/kg/week IgE antagonist for every IU/ml baseline free IgE in the patient's serum for a period of at least about 14 days, followed by a maintenance dose of the IgE antagonist averaging about 0.00008 to 0.0024 mg/kg/week IgE antagonist for every IU/ml baseline free IgE in the patient's serum, wherein the maintenance dose is less than about one third of the loading dose.

Also provided is a method for treating allergic disease, including allergic rhinitis and allergic asthma, comprising administering a loading dose of an IgE antagonist for a period of at least about 14 days, followed by a maintenance dose of the IgE antagonist that maintains the patient's serum free IgE concentration at a level no greater than about 600 ng/ml, wherein the loading dose exceeds the maintenance dose by at least about three fold in units of mg/kg/week IgE antagonist for every IU/ml baseline free IgE in the patient's serum.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 is a graph depicting serum concentrations of free IgE in patients undergoing anti-IgE antibody therapy. The values shown are mean ± standard deviation (SD) free IgE concentrations predose and two hours post-dose on days 0, 7, 14, 28, and 42 of the study for the twelve patients for whom free IgE data was available. The means and standard deviations were calculated on the log-transformed free IgE concentrations.

Figs. 2A-C are graphs depicting expression of endogenous IgE and FcεRI on basophils of donors undergoing anti-IgE antibody therapy. Figs. 2A-C show the results for 12 treated patients and 2 controls and the data were generated using the acetate buffer "stripping" technique. Fig. 2A shows the results for endogenous IgE antibody. Fig. 2B displays the results of IgE measurements for cells fully sensitized and therefore plots total receptor densities. Fig. 2C displays the BPO-specific IgE densities and therefore plots unoccupied receptor densities. As explained in greater detail in Example 1 below, the stars represent calculated maximums for measurements where the IgE level was not detectable by the total IgE radioimmunoassay. The open symbols indicate the controls and closed symbols indicate the treated patients.

Figs. 3A-B are graphs depicting the levels of basophil IgE with and without sensitization as determined by flow cytometry. Fig. 3A shows the results for endogenous IgE expression (without sensitization) for 12 patients on therapy at three time points and Fig. 3B shows similar data for cells first sensitized with IgE myeloma antibody.

Figs. 4A-B are graphs depicting flow cytometric measurements of endogenous IgE and FcεRI expression during the first 2 weeks of therapy for 3 patients with the highest serum levels of IgE at enrollment. Fig. 4A shows the results for IgE expression without prior sensitization (open symbols) and with prior sensitization (closed symbols) for three different subjects. Fig. 4B shows the results for FcεRI expression as determined by the binding of the anti-FcεRIα antibody, 29C6. Values of zero in Fig. 4A represent undetectable FcεRI expression levels as compared to background signal produced by FITC-conjugated normal goat IgG. Values for irrelevant IgG controls in Fig. 4B were 3-5. The dosing regimen indicated by the arrows is described in Example 1 below.

Fig. 5 is a graph depicting the histamine release response of basophils challenged with 3 secretagogues before therapy and 3 months into therapy. The left portion of the figure shows the anti-IgE antibody dose response curves at the two time points; pre-therapy (represented as open circles) and the 3 month time point (represented as closed circles). The right side histograms show the responses to FMLP and dust mite antigen (D. farinae at 10 PNU/ml). The box plots show the median ±25% of the median at the two time points.

DETAILED DESCRIPTION OF THE INVENTION

A. DEFINITIONS

The terms "allergy" and "atopy" and all their grammatical variants are used synonymously herein to refer to any disease mediated by a Type I (Gell & Coombs classification) hypersensitivity reaction, including allergic rhinitis, atopic dermatitis, anaphylaxis, allergic asthma.

The term "asthma" as used herein refers to a lung disease characterized by airway obstruction that is reversible (although not entirely in some patients) either spontaneously or with treatment, airway

inflammation, and increased airway resp nsiveness to a variety of stimuli. "Allergic asthma" as used herein refers to an asthmatic response to inhalation of an antigen to which the patient is sensitive.

The term "early asthmatic response" (EAR) as used herein refers to an asthmatic response to an antigen within about two hours of exposure. The term "late asthmatic response" (LAR) as used herein refers to an asthmatic response to an antigen within about two to eight hours after exposure.

The term "allergic rhinitis" as used herein refers to any allergen-induced nasal symptoms, including itching, sneezing, nasal congestion, nasal discharge, and symptoms associated with nasal mucosal inflammation.

The term "IgE antagonist" as used herein refers to a substance which inhibits the biological activity of IgE. Such antagonists include but are not limited to anti-IgE antibodies, IgE receptors, anti-IgE receptor antibodies, variants of IgE antibodies, ligands for the IgE receptors, and fragments thereof. Antibody antagonists may be of the IgA, IgD, IgG, or IgM class. Variant IgE antibodies typically have amino acid substitutions or deletions at one or more amino acid residues. Ligands for IgE receptors include but are not limited to IgE and anti-receptor antibodies, and fragments thereof capable of binding to the receptors, including amino acid substitution and deletion variants, and cyclized variants.

In general, in some embodiments of the invention, IgE antagonists act by blocking the binding of IgE to its receptors on B cells, mast cells, or basophils, either by blocking the receptor binding site on the IgE molecule or blocking its receptors. Additionally, in some embodiments of the invention, IgE antagonists act by binding soluble IgE and thereby removing it from circulation. The IgE antagonists of the invention can also act by binding to membrane-bound IgE on B cells, thereby eliminating clonal populations of B cells. The IgE antagonists of the instant invention can also act by inhibiting IgE production. Preferably, the IgE antagonists of the instant invention do not result in histamine release from mast cells or basophils.

The term "treatment" as used herein denotes therapy or prophylaxis that prevents or ameliorates symptoms of a disorder or responsive pathologic physiological condition.

The term "mg/kg/week IgE antagonist for every IU/ml baseline free IgE in the patient's serum" as used herein is defined as a drug dosage unit expressed in terms of an average amount of IgE antagonist in milligrams administered per kilogram of patient body weight per week for every International Unit of baseline free IgE per milliliter of patient serum. An International Unit of IgE is defined as 2.4 ng/ml. The per week term does not necessarily indicate weekly administration, but rather the average weekly amount of drug received calculated by dividing the amount of drug received over a given treatment period by the number of weeks in the treatment period.

"Polypeptide" as used herein refers generally to peptides and proteins having at least about two amino acids.

The term "free IgE" as used herein refers to IgE not complexed to a binding partner, such an anti-IgE antibody. The term "total IgE" as used herein refers to the measurement of free IgE and IgE complexed to a binding partner, such as an anti-IgE antibody. The terms "baseline IgE" and "baseline free IgE" as used herein refer to the level of free IgE in a patient's serum before treatment with an IgE antagonist.

The term "polyol" as used herein denotes a hydrocarbon including at least two hydroxyls bonded to carbon atoms, such as polyethers (e.g. polyethylene glycol), trehalose, and sugar alcohols (such as mannitol).

The term "polyether" as used herein denotes a hydrocarbon c ntaining at least three ether bonds. Polyethers can include other functional groups. Polyethers useful for practicing the invention include polyethylene glycol (PEG).

B. GENERAL METHODS

The present invention arises from the inventors' discovery that treatment of atopic patients with IgE antagonist results in the down regulation of the high affinity IgE receptor (FcεRI) on the basophils from the treated patients. Based on these surprising and unexpected results, the inventors determined that the down regulation of the FcεRI produced by an unusually high loading dose of IgE antagonist significantly reduces the level of IgE antagonist that would need to be maintained in order to block IgE-mediated allergic responses in atopic patients. Accordingly, the inventors designed and developed methods for treating allergic disease with IgE antagonists wherein a loading dose of IgE antagonist that is significantly higher than that ordinarily designed to reduce the time to attain a target serum drug concentration is used to down-regulate IgE receptor on basophils (basophil stripping), followed by a maintenance dose regimen providing significantly lower drug amounts and/or fewer drug administrations than would a typical maintenance regimen that is designed to maintain equilibrium at the target serum drug concentration attained with the ordinary loading dose. The methods of the invention advantageously reduce the cost and other burdens affecting the treatment of allergy with IgE antagonists by significantly reducing the amount of drug and/or frequency of treatment required.

1. IgE Antagonists

IgE antagonists suitable for use in the methods of the invention include anti-IgE antibodies, IgE-binding proteins, soluble IgE receptors, anti-IgE receptor antibodies, IgE variants and other IgE receptor binding competitors.

Polyclonal antibodies to IgE generally are raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of IgE and an adjuvant. It can be useful to conjugate IgE or a fragment containing the target amino acid sequence from the Fc region of IgE to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, $SOCl_2$, or $R^1N = C = NR$, where R and R^1 are different alkyl groups.

Animals ordinarily are immunized against the cells or immunogenic conjugates or derivatives by combining 1 mg or 1 μg of IgE with Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 1/5 to 1/10 the original amount of conjugate in Freund's incomplete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later, animals are bled and the serum is assayed for anti-IgE titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with a conjugate of the same IgE, but conjugated to a different protein and/or through a different cross-linking agent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum can be used to enhance the immune response.

Mon clonal antibodies are prepared by recovering spleen cells from immunized animals and immortalizing the cells in conventional fashion, e.g. by fusion with myeloma cells or by Epstein-Barr (EB)-virus transformation and screening for clones expressing the desired antibody. The hybridoma technique described originally by Koehler and Milstein, *Eur. J. Immunol.*, 6: 511 (1976) and also described by Hammerling *et al.*, in: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., pp. 563-681 (1981) has been widely applied to produce hybrid cell lines that secrete high levels of monoclonal antibodies against many specific antigens.

The hybrid cell lines can be maintained *in vitro* in cell culture media. The cell lines producing the antibodies can be selected and/or maintained in a composition comprising the continuous cell line in hypoxanthine-aminopterinthymidine (HAT) medium. In fact, once the hybridoma cell line is established, it can be maintained on a variety of nutritionally adequate media. Moreover, the hybrid cell lines can be stored and preserved in any number of conventional ways, including freezing and storage under liquid nitrogen. Frozen cell lines can be revived and cultured indefinitely with resumed synthesis and secretion of monoclonal antibody.

The secreted antibody is recovered from tissue culture supernatant by conventional methods such as precipitation, ion-exchange chromatography, affinity chromatography, or the like. The antibodies described herein are also recovered from hybridoma cell cultures by conventional methods for purification of IgG or IgM, as the case may be, that heretofore have been used to purify these immunoglobulins from pooled plasma, e.g., ethanol or polyethylene glycol precipitation procedures. The purified antibodies are sterile-filtered.

While mouse monoclonal antibodies are routinely used, the invention is not so limited; in fact, human antibodies can be used. Such antibodies can be obtained, for example, by using human hybridomas (Cote *et al., Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985)). In fact, according to the invention, techniques developed for the production of chimeric antibodies (Cabilly *et al.*, U.S. 4,816,567, Morrison *et al., Proc. Natl. Acad. Sci.* **81**: 6851 (1984); Boulianne *et al., Nature* **312**: 643-646 (1984); Neuberger *et al., Nature*, **312**: 604 (1984); Neuberger *et al., Nature* **314**: 268-270 (1985); Takeda *et al., Nature* **314**: 452 (1985); EP 184,187; EP 171,496; EP 173,494; PCT WO 86/01533; Shaw *et al., J. Nat. Canc. Inst.* **80**: 1553-1559 (1988); Morrison, *Science* **229**: 1202-1207 (1985); Oi *et al., BioTechniques*, **4**: 214 (1986)) by coupling an animal antigen-binding variable domain to a human constant domain can be used; such antibodies are within the scope of this invention. The term "chimeric" antibody is used herein to describe a polypeptide comprising at least the antigen binding portion of an antibody molecule linked to at least part of another protein (typically an immunoglobulin constant domain).

In one embodiment, such chimeric antibodies contain about one third rodent (or other non-human species) sequence and thus are capable of eliciting a significant anti-globulin response in humans. For example, in the case of the murine anti-CD3 antibody OKT3, much of the resulting anti-globulin response is directed against the variable region rather than the constant region (Jaffers *et al., Transplantation* **41**: 572-578 (1986)).

Humanized antibodies are used to reduce or eliminate any anti-globulin immune response in humans. In practice, humanized antibodies are typically human antibodies in which some amino acid residues from the

complementarity determining regions (CDRs), the hypervariable regions in the variable domains which are directly involved with formation of the antigen-binding site, and possibly some amino acids from the framework regions (FRs), the regions of sequence that are somewhat conserved within the variable domains, are substituted by residues from analogous sites in rodent antibodies. The construction of humanized antibodies is described in Riechmann *et al., Nature* **332**: 323-327 (1988), Queen *et al., Proc. Natl. Acad. Sci. USA* **86**: 10029-10033 (1989), Co *et al., Proc. Natl. Acad. Sci. USA* **88**: 2869-2873 (1991), Gorman *et al., Proc. Natl. Acad. Sci.* **88**: 4181-4185 (1991), Daugherty *et al., Nucleic Acids Res.* **19**: 2471-2476 (1991), Brown *et al., Proc. Natl. Acad. Sci. USA* **88**: 2663-2667 (1991), Junghans *et al., Cancer Res.* **50**: 1495-1502 (1990), Fendly *et al., Cancer Res.* **50**: 1550-1558 (1990) and in PCT applications WO 89/06692 and WO 92/22653.

In some cases, substituting CDRs from rodent antibodies for the human CDRs in human frameworks is sufficient to transfer high antigen binding affinity (Jones *et al., Nature* **321**: 522-525 (1986); Verhoeyen *et al., Science* **239**: 1534-1536 (1988)) whereas in other cases it is necessary to additionally replace one (Riechmann *et al., supra)* or several (Queen *et al., supra*) FR residues. See also Co *et al., supra.*

The invention also encompasses the use of human antibodies produced in transgenic animals. In this system, DNA encoding the antibody of interest is isolated and stably incorporated into the germ line of an animal host. The antibody is produced by the animal and harvested from the animal's blood or other body fluid. Alternatively, a cell line that expresses the desired antibody can be isolated from the animal host and used to produce the antibody in vitro, and the antibody can be harvested from the cell culture by standard methods.

Anti-IgE antibody fragments can also be used in the methods of the invention. Any fragment of an anti-IgE antibody capable of blocking or disrupting IgE interaction with its receptor is suitable for use herein.

Suitable anti-IgE antibody fragments can be obtained by screening combinatorial variable domain libraries for DNA capable of expressing the desired antibody fragments. These techniques for creating recombinant DNA versions of the antigen-binding regions of antibody molecules which bypass the generation of monoclonal antibodies, are encompassed within the practice of this invention. One typically extracts antibody-specific messenger RNA molecules from immune system cells taken from an immunized animal, transcribes these into complementary DNA (cDNA), and clones the cDNA into a bacterial expression system. "Phage display" libraries are an example of such techniques. One can rapidly generate and screen great numbers of candidates for those that bind the antigen of interest. Such IgE-binding molecules are specifically encompassed within the term "antibody" as it is defined, discussed, and claimed herein.

In some embodiments, the IgE antagonist binds to the IgE receptor binding site on the IgE molecule, thereby preventing the IgE molecule from binding to the IgE receptor. In other embodiments, the IgE antagonist is an anti-IgE antibody that binds to the IgE receptor binding site on the IgE molecule. Preferred embodiments of the invention include methods using the monoclonal antibodies MAE11, MAE13, MAE15, and MAE17 disclosed in copending application WO 93/04173. Particularly preferred are embodiments using humanized versions of MAE11, including variant 8b disclosed in Table 9 on page 68 of WO 93/04173. Variant 8b is also described as humanized variant 12 of MAE11 in Presta et al., supra, and is identical to the

rhuMAb-E25 antibody described in Example 1 below. Other preferred embodiments of the invention use antibodies capable of binding to (B cell) membrane-bound IgE, such as rhuMAb-E25. In some embodiments, the methods of the invention use antibodies that suppress serum IgE levels by clonal deletion of IgE-producing B cells or inhibit IgE production by other mechanisms.

In a further embodiment of the invention, soluble IgE receptor can be used as the IgE antagonist. Soluble receptors suitable for use herein include, for example, molecules comprising the IgE binding site in the extracellular domain (exodomain) of the FcεRI α-chain. The α-chain of FcεRI can be genetically modified such that the exodomain is secreted as a soluble protein in a recombinant expression system according to the method of Blank *et al., J. Biol. Chem.*, **266**: 2639-2646 (1991) or Qu *et al., J. Exp. Med.*, **167**: 1195.

The invention also encompasses the use of IgE-binding peptides in addition to anti-IgE antibodies and soluble receptor. Any IgE-binding peptide capable of disrupting or blocking the interaction between IgE and its receptors is suitable for use herein.

In addition to IgE antagonists which interfere with IgE/receptor interaction by binding to IgE, such as anti-IgE antibodies, fragments thereof, soluble IgE receptor and other IgE-binding peptides described above, the invention encompasses the use of IgE antagonists which disrupt IgE/receptor interaction by competing with IgE for binding to its receptor, thereby lowering the available IgE receptor.

IgE variants are an example of a receptor-binding competitor that is suitable for use in the methods of the invention. IgE variants are forms of IgE possessing an alteration, such as an amino acid substitution or substitutions and/or an amino acid deletion or deletions, wherein the altered IgE molecule is capable of competing with IgE for binding to its receptors.

Fragments of IgE variants are also suitable for use herein. Any fragment of an IgE variant capable of competing with IgE for binding to its receptors can be used in the methods of the invention.

The invention also encompasses the use of IgE receptor-binding peptides in addition to IgE variants and fragments thereof. Any IgE receptor-binding peptide capable of disrupting or blocking the interaction between IgE and its receptors is suitable for use herein.

2. Therapeutic Compositions Containing IgE Antagonist

In general, the formulations of the subject invention can contain other components in amounts not detracting from the preparation of stable forms and in amounts suitable for effective, safe pharmaceutical administration. For example, other pharmaceutically acceptable excipients well known to those skilled in the art can form a part of the subject compositions. These include, for example, salts, various bulking agents, additional buffering agents, chelating agents, antioxidants, cosolvents and the like; specific examples of these include tris-(hydroxymethyl)aminomethane salts ("Tris buffer"), and disodium edetate.

In one embodiment of the invention, IgE antagonist formulations comprise a buffer, a salt, optionally, a polyol, and optionally, a preservative.

One exemplary formulation of the invention is a liquid formulation of about 1-100 mg/ml IgE antagonist in 10 mM acetate buffer, pH 5.0-6.5, 100-200 mM sodium chloride, and about 0.01% polysorbate 20, more preferably about 5 mg/ml IgE antagonist in 10 mM acetate buffer, pH 5.2, 142 mM sodium chloride, and 0.01% polysorbate 20. In other embodiments of the invention, the formulation may be freeze-dried and

reconstituted for administration. For example, anti-IgE antibody can be formulated at about 25 mg/ml in 5 mM histidine, pH 6.0, and 88 mM sucrose, freeze-dried, and reconstituted in water to 100 mg/ml antibody for administrati n. Mixed sugars can also be used, such as a combination of sucrose and mannitol, etc.

In one embodiment, the invention provides for the treatment of allergic diseases, including allergic asthma diseases by administration of IgE antagonist to the respiratory tract. The invention contemplates formulations comprising an IgE antagonist for use in a wide variety of devices that are designed for the delivery of pharmaceutical compositions and therapeutic formulations to the respiratory tract. In one aspect of the present invention, an IgE antagonist is administered in aerosolized or inhaled form. The IgE antagonist, combined with a dispersing agent, or dispersant, can be administered in an aerosol formulation as a dry powder or in a solution or suspension with a diluent.

Suitable dispersing agents are well known in the art, and include but are not limited to surfactants and the like. Surfactants are generally used in the art to reduce surface induced aggregation of protein caused by atomization of the solution forming the liquid aerosol. Examples of such surfactants include polyoxyethylene fatty acid esters and alcohols, and polyoxyethylene sorbitan fatty acid esters. Amounts of surfactants used will vary, being generally within the range of about 0.001 to 4% by weight of the formulation. In a specific aspect, the surfactant is polyoxyethylene sorbitan monooleate or sorbitan trioleate.

The liquid aerosol formulations contain the IgE antagonist and a dispersing agent in a physiologically acceptable diluent. The dry powder aerosol formulations of the present invention consist of a finely divided solid form of the IgE antagonist and a dispersing agent, and optionally a bulking agent, such as lactose, sorbitol, sucrose, or mannitol, and the like, to facilitate dispersal of the powder. With either the liquid or dry powder aerosol formulation, the formulation must be aerosolized. That is, it must be broken down into liquid or solid particles in order to ensure that the aerosolized dose actually reaches the bronchii and/or alveoli, as desired. For example, in the methods for treatment of asthma provided herein, it is preferable to deliver aerosolized IgE antagonist to the bronchii. In other embodiments, such as the present methods for treating adult respiratory distress syndrome, it is preferably to deliver aerosolized IgE antagonist to the alveoli. In general the mass median dynamic diameter will be 5 micrometers (μm) or less in order to ensure that the drug particles reach the lung bronchii or alveoli (Wearley, L.L., *Crit. Rev. in Ther. Drug Carrier Systems* 8:333 (1991)).

With regard to construction of the delivery device, any form of aerosolization known in the art, including but not limited to nebulization, atomization or pump aerosolization of a liquid formulation, and aerosolization of a dry powder formulation, can be used in the practice of the invention. A delivery device that is uniquely designed for administration of solid formulations is envisioned. Often, the aerosolization of a liquid or a dry powder formulation will require a propellent. The propellent can be any propellant generally used in the art. Examples of useful propellants include chlorofluorocarbons, hydrofluorocarbons, hydochlorofluorocarbons, and hydrocarbons, including triflouromethane, dichlorodifluoromethane, dichlorotetrafluoroethanol, and 1,1,1,2-tetrafluoroethane, and combinations thereof.

In a preferred aspect of the invention, the device for aerosolization is a metered dose inhaler. A metered dose inhaler provides a specific dosage when administered, rather than a variable dose depending on

administration. Such a metered dose inhaler can be used with either a liquid or a dry powder aerosol formulation.

Systems of aerosol delivery, such as the pressurized metered dose inhaler and the dry powder inhaler are disclosed in Newman, S.P., *Aerosols and the Lung*, Clarke, S.W. and Davia, D. editors, pp. 197-22 and can be used in connection with the present invention.

Additional pharmaceutical methods may be employed to control the duration of action of the antagonists of this invention. The antagonists also may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in Remington's *Pharmaceutical Sciences*, 16th edition, Osol, A., ed., 1980).

3. Therapeutic Administration of IgE Antagonists

In general, the invention provides for methods for treatment or prophylaxis of allergic disease in a patient by administering a loading dose of an IgE antagonist that is sufficient to reduce the patient's serum IgE level for a period of time that is sufficient to significantly suppress the expression of FcεRI receptor on the basophil cell surface, such that a substantially reduced maintenance regimen of IgE antagonist is sufficient to prevent the reoccurrence of allergic symptoms. The basophil IgE receptor suppression resulting from the unusually high loading dose reduces the receptor density available to trigger an allergic cascade by interaction with allergen-specific IgE, and enables the treating physician to use a significantly lower maintenance dose to prevent basophils from reaching the threshold level of allergen-specific serum IgE sensitization required for degranulation.

In some embodiments, the loading regimen and/or maintenance regimen dosing amounts are determined according to the baseline serum IgE level of the patient. Patient serum IgE levels are typically assayed by standard ELISA techniques well known in the art. Total serum IgE can be measured by commercially available assays, such as Abbott Laboratories' Total IgE assay. Free IgE, e.g., IgE not bound to antibody can be measured by a capture type assay in which, for example, IgE receptor is bound to a solid support. IgE complexed to an anti-IgE antibody which binds at or near the site on IgE which binds to the receptor will be blocked from binding the receptor, and thus only free or unbound IgE can react with the receptor bound to the solid support in this assay. An anti-IgE antibody which recognizes IgE even when the IgE is bound to its receptor can be used to detect the IgE captured by the receptor on the solid support. This anti-IgE antibody can be labeled with any of a variety of reporter systems, such as alkaline phosphatase, etc.

In some embodiments, the methods of the invention provide for treatment of allergic disease by administering a loading dose of an IgE antagonist for a period of at least about 14 days, or at least about 28 days, or at least about 42 days, sufficient to reduce the patient's average serum free IgE level to a level no greater than 50 ng/ml, or no greater than 30 ng/ml, or no greater than 16 ng/ml, or no greater than 10 ng/ml, or no greater than 6 ng/ml, at the end of the loading period, followed by a maintenance dose of the IgE antagonist averaging about 0.00008 to 0.0024 mg/kg/week, or about 0.00025 t 0.0024 mg/kg/week, or about

0.0008 to 0.0024 mg/kg/week, of IgE antagonist for every IU/ml baseline free IgE in the patient's serum. In other embodiments, the maintenance dose averages about 0.00075 to 0.0024 mg/kg/week, or about 0.00125 to 0.0024 mg/kg/week, or about 0.00175 to 0.0024 mg/kg/week, of IgE antagonist for every IU/ml baseline free IgE in the patient's serum. Preferably, the maintenance dose is at least about three fold lower, or at least about six fold lower, or at least about 12 fold lower, or at least about 25 fold lower, or at least about 50 fold lower, than the loading dose in units of mg/kg/week for every IU/ml baseline free IgE in the patient's serum. In these embodiments, the patient's serum free IgE response to the loading regimen can be determined by assaying the patient's serum free IgE according to the methods for baseline serum free IgE assay described above.

In the practice of the foregoing methods, the patient's serum IgE level can be reduced to the prescribed level at the end of the loading period by administering for the prescribed loading period a loading dose of at least about 0.003 mg/kg/week of the IgE antagonist, or at least about 0.007 mg/kg/week of the IgE antagonist, or at least about 0.021 mg/kg/week of the IgE antagonist, or about 0.003 to 0.030 mg/kg/week of the IgE antagonist, or about 0.007 to 0.021 mg/kg/week of the IgE antagonist, for every IU/ml baseline free IgE in the patient's serum. However, the invention also encompasses the use of other loading doses that are sufficient to strip basophils of IgE receptor such that maintenance dose levels provided in the methods are sufficient to substantially maintain IgE receptor suppression and avoid reoccurrence of symptoms. In some embodiments, the foregoing methods are used to treat allergic rhinitis or allergic asthma.

In other embodiments, the methods of the invention provide for the treatment of allergic disease by administering a loading dose of an IgE antagonist averaging at least about 0.003 mg/kg/week IgE antagonist, or at least about 0.007 mg/kg/week IgE antagonist, or at least about 0.021 mg/kg/week IgE antagonist, or about 0.003 to 0.030 mg/kg/week IgE antagonist, or about 0.007 to 0.021 mg/kg/week IgE antagonist, for every IU/ml baseline free IgE in the patient's serum for a period of at least about 14 days, or about 14 days to 56 days, or about 21 days to 56 days, or about 28 days to 56 days, or about 35 days to 56 days, or about 42 days to 56 days, or about 49 days to 56 days, followed by a maintenance dose of the IgE antagonist averaging about 0.00008 to 0.0024 mg/kg/week IgE antagonist , or about 0.00025 to 0.0024 mg/kg/week IgE antagonist, or about 0.0008 to 0.0024 mg/kg/week IgE antagonist, for every IU/ml baseline free IgE in the patient's serum. In other embodiments, the maintenance dose averages about 0.00075 to 0.0024 mg/kg/week, or about 0.00125 to 0.0024 mg/kg/week, or about 0.00175 to 0.0024 mg/kg/week, of IgE antagonist for every IU/ml baseline free IgE in the patient's serum. Preferably, the maintenance dose is at least about three fold lower, or at least about six fold lower, or at least about 12 fold lower, or at least about 25 fold lower, or at least about 50 fold lower, than the loading dose in units of mg/kg/week for every IU/ml baseline free IgE in the patient's serum. In some embodiments, these methods are used to treat allergic rhinitis or allergic asthma.

The invention additionally encompasses methods for treating allergic disease comprising administering a loading dose of an IgE antagonist for a period of at least about 14 days, or about 28 to 56 days, or about 42 to 56 days, followed by a maintenance dose of the IgE antagonist that maintains the patient's serum free IgE concentration at a level no greater than about 600 ng/ml, or no greater than about 300 ng/ml, or no greater than about 150 ng/ml, or no greater than about 75 ng/ml, or no greater than about 50 ng/ml, wherein

the loading dose exceeds the maintenance dose by at least about three fold, or by at least about six fold, or by at least about 12 fold, or by at least about 25 fold, or by at least about 50 fold, in units of mg/kg/week IgE antagonist for every IU/ml baseline free IgE in the patient's serum. In some embodiments, these methods are used to treat allergic rhinitis or allergic asthma.

In these embodiments, a typical loading dose used is at least about 0.003 mg/kg/week IgE antagonist, or at least about 0.007 mg/kg/week IgE antagonist, or at least about 0.021 mg/kg/week IgE antagonist, or about 0.003 to 0.030 mg/kg/week IgE antagonist, or about 0.007 to 0.021 mg/kg/week IgE antagonist, for every IU/ml baseline free IgE in the patient's serum. However, the invention also encompasses the use of other loading doses that are sufficient to strip basophils of IgE receptor such that maintenance dose levels which provide the serum free IgE levels recited in the methods are sufficient to substantially maintain IgE receptor suppression and avoid reoccurrence of symptoms. Suitable maintenance dosing for practice of these methods include maintenance doses of about 0.00008 to 0.0024 mg/kg/week IgE antagonist, or about 0.00025 to 0.0024 mg/kg/week IgE antagonist, or about 0.0008 to 0.0024 mg/kg/week IgE antagonist, for every IU/ml baseline free IgE in the patient's serum. In other embodiments, the maintenance dose averages about 0.00075 to 0.0024 mg/kg/week, or about 0.00125 to 0.0024 mg/kg/week, or about 0.00175 to 0.0024 mg/kg/week, of IgE antagonist for every IU/ml baseline free IgE in the patient's serum. The patient's serum free IgE response to the maintenance regimen can be determined by assaying serum free IgE according to the methods for baseline serum free IgE assay described above.

The invention further provides methods for reducing reactivity to intrabronchial allergen challenge in a patient comprising administering a loading dose of an IgE antagonist for a period of at least about 14 days, or at least about 28 days, or at least about 42 days, sufficient to reduce the patient's average serum free IgE level to a level no greater than 50 ng/ml, or no greater than 30 ng/ml, or no greater than 16 ng/ml, or no greater than 10 ng/ml, or no greater than 6 ng/ml, at the end of the loading period, followed by a maintenance dose of the IgE antagonist averaging about 0.00008 to 0.0024 mg/kg/week, or about 0.00025 to 0.0024 mg/kg/week, or about 0.0008 to 0.0024 mg/kg/week, of IgE antagonist for every IU/ml IgE in the patient's serum. In other embodiments, the maintenance dose averages about 0.00075 to 0.0024 mg/kg/week, or about 0.00125 to 0.0024 mg/kg/week, or about 0.00175 to 0.0024 mg/kg/week, of IgE antagonist for every IU/ml baseline free IgE in the patient's serum. Preferably, the maintenance dose is at least about three fold lower, or at least about six fold lower, or at least about 12 fold lower, or at least about 25 fold lower, or at least about 50 fold lower, than the loading dose in units of mg/kg/week for every IU/ml baseline free IgE in the patient's serum. In these embodiments, the patient's serum free IgE response to the loading regimen can be determined by assaying the patient's serum free IgE according to the methods for baseline serum free IgE assay described above. The patient's reactivity to intrabronchial allergen challenge can be determined according to the methods of Naclerio *et al.*, "IN VIVO METHODS FOR THE STUDY OF ALLERGY: Mucosal tests, techniques and interpretations" *Allergy Principles & Practice*, Middleton et al., eds, pps. 613-627 (1992), or as described in Example 2 below.

In the practice of the foregoing methods for reducing bronchial hyperreactivity, the patient's serum IgE level can be reduced to the prescribed level at the end of the loading period by administering for the

prescribed loading period a l ading dose of at least about 0.003 mg/kg/week of the IgE antagonist, or at least about 0.007 mg/kg/week of the IgE antagonist, or at least about 0.021 mg/kg/week of the IgE antagonist, or about 0.003 to 0.030 mg/kg/week f the IgE antagonist, or about 0.007 to 0.021 mg/kg/week of the IgE antagonist, for every IU/ml baseline free IgE in the patient's serum. However, the invention also encompasses the use of other loading doses that are sufficient to strip basophils of IgE receptor such that maintenance dose levels provided in the methods are sufficient to substantially maintain IgE receptor suppression and avoid reoccurrence of symptoms.

The invention additionally provides methods for reducing reactivity to intrabronchial allergen challenge in a patient comprising administering a loading dose of an IgE antagonist averaging at least about 0.003 mg/kg/week IgE antagonist, or at least about 0.007 mg/kg/week IgE antagonist, or at least about 0.021 mg/kg/week IgE antagonist, or about 0.003 to 0.030 mg/kg/week IgE antagonist, or about 0.007 to 0.021 mg/kg/week IgE antagonist, for every IU/ml baseline free IgE in the patient's serum for a period of at least about 14 days, or about 14 days to 56 days, or about 21 days to 56 days, or about 28 days to 56 days, or about 35 days to 56 days, or about 42 days to 56 days, or about 49 days to 56 days, followed by a maintenance dose of the IgE antagonist averaging about 0.00008 to 0.0024 mg/kg/week IgE antagonist , or about 0.00025 to 0.0024 mg/kg/week IgE antagonist, or about 0.0008 to 0.0024 mg/kg/week IgE antagonist, for every IU/ml baseline free IgE in the patient's serum. In other embodiments, the maintenance dose averages about 0.00075 to 0.0024 mg/kg/week, or about 0.00125 to 0.0024 mg/kg/week, or about 0.00175 to 0.0024 mg/kg/week, of IgE antagonist for every IU/ml baseline free IgE in the patient's serum. Preferably, the maintenance dose is at least about three fold lower, or at least about six fold lower, or at least about 12 fold lower, or at least about 25 fold lower, or at least about 50 fold lower, than the loading dose in units of mg/kg/week for every IU/ml baseline free IgE in the patient's serum. The patient's reactivity to intrabronchial allergen challenge can be determined as described above.

The invention also provides methods for reducing reactivity to intrabronchial allergen challenge in a patient comprising administering a loading dose of an IgE antagonist for a period of at least about 14 days, or about 28 to 56 days, or about 42 to 56 days, followed by a maintenance dose of the IgE antagonist that maintains the patient's serum free IgE concentration at a level no greater than about 600 ng/ml, or no greater than about 300 ng/ml, or no greater than about 150 ng/ml, or no greater than about 75 ng/ml, or no greater than about 50 ng/ml, wherein the loading dose exceeds the maintenance dose by at least about three fold, or by at least about six fold, or by at least about 12 fold, or by at least about 25 fold, or by at least about 50 fold, in units of mg/kg/week IgE antagonist for every IU/ml baseline free IgE in the patient's serum. The patient's reactivity to intrabronchial allergen challenge can be determined as described above. The patient's serum free IgE response to the maintenance regimen can be determined by assaying serum free IgE according to the methods for baseline serum free IgE assay described above.

In these embodiments, a typical loading dose used is at least about 0.003 mg/kg/week IgE antagonist, or at least about 0.007 mg/kg/week IgE antagonist, or at least about 0.021 mg/kg/week IgE antagonist, or about 0.003 to 0.030 mg/kg/week IgE antagonist, or about 0.007 to 0.021 mg/kg/week IgE antagonist, for every IU/ml baseline free IgE in the patient's serum. However, the invention also encompasses the use of other

loading doses that are sufficient to strip basophils of IgE receptor such that maintenance dose levels which provide the serum free IgE levels recited in the methods are sufficient to substantially maintain IgE receptor suppression and avoid reoccurrence of symptoms. Suitable maintenance dosing for practice of these methods include maintenance doses of about 0.00008 to 0.0024 mg/kg/week IgE antagonist, or about 0.00025 to 0.0024 mg/kg/week IgE antagonist, or about 0.0008 to 0.0024 mg/kg/week IgE antagonist, for every IU/ml baseline free IgE in the patient's serum. In other embodiments, the maintenance dose averages about 0.00075 to 0.0024 mg/kg/week, or about 0.00125 to 0.0024 mg/kg/week, or about 0.00175 to 0.0024 mg/kg/week, of IgE antagonist for every IU/ml baseline free IgE in the patient's serum.

The invention further provides methods for reducing reactivity to intranasal allergen challenge in a patient comprising administering a loading dose of an IgE antagonist for a period of at least about 14 days, or at least about 28 days, or at least about 42 days, sufficient to reduce the patient's average serum free IgE level to a level no greater than 50 ng/ml, or no greater than 30 ng/ml, or no greater than 16 ng/ml, or no greater than 10 ng/ml, or no greater than 6 ng/ml, at the end of the loading period, followed by a maintenance dose of the IgE antagonist averaging about 0.00008 to 0.0024 mg/kg/week, or about 0.00025 to 0.0024 mg/kg/week, or about 0.0008 to 0.0024 mg/kg/week, of IgE antagonist for every IU/ml IgE in the patient's serum. In other embodiments, the maintenance dose averages about 0.00075 to 0.0024 mg/kg/week, or about 0.00125 to 0.0024 mg/kg/week, or about 0.00175 to 0.0024 mg/kg/week, of IgE antagonist for every IU/ml baseline free IgE in the patient's serum. Preferably, the maintenance dose is at least about three fold lower, or at least about six fold lower, or at least about 12 fold lower, or at least about 25 fold lower, or at least about 50 fold lower, than the loading dose in units of mg/kg/week for every IU/ml baseline free IgE in the patient's serum. In these embodiments, the patient's serum free IgE response to the loading regimen can be determined by assaying the patient's serum free IgE according to the methods for baseline serum free IgE assay described above. The patient's reactivity to intranasal allergen challenge can be determined according to the methods of Naclerio *et al.*, "IN VIVO METHODS FOR THE STUDY OF ALLERGY: Mucosal tests, techniques and interpretations" *Allergy Principles & Practice*, Middleton et al., eds, pps. 595-613 (1992) or as described in Example 2 below.

In the practice of the foregoing methods for reducing reactivity to intranasal allergen challenge, the patient's serum IgE level can be reduced to the prescribed level at the end of the loading period by administering for the prescribed loading period a loading dose of at least about 0.003 mg/kg/week of the IgE antagonist, or at least about 0.007 mg/kg/week of the IgE antagonist, or at least about 0.021 mg/kg/week of the IgE antagonist, or about 0.003 to 0.030 mg/kg/week of the IgE antagonist, or about 0.007 to 0.021 mg/kg/week of the IgE antagonist, for every IU/ml baseline free IgE in the patient's serum. However, the invention also encompasses the use of other loading doses that are sufficient to strip basophils of IgE receptor such that maintenance dose levels provided in the methods are sufficient to substantially maintain IgE receptor suppression and avoid reoccurrence of symptoms.

The invention additionally provides methods for reducing reactivity to intranasal allergen challenge in a patient comprising administering a loading dose of an IgE antagonist averaging at least about 0.003 mg/kg/week IgE antag nist, or at least about 0.007 mg/kg/week IgE antagonist, or at least about 0.021

mg/kg/week IgE antagonist, or about 0.003 to 0.030 mg/kg/week IgE antagonist, or about 0.007 to 0.021 mg/kg/week IgE antag nist, for every IU/ml baseline free IgE in the patient's serum for a period of at least about 14 days, r about 14 days to 56 days, or about 21 days to 56 days, or about 28 days to 56 days, or about 35 days to 56 days, or about 42 days to 56 days, or about 49 days to 56 days, followed by a maintenance dose of the IgE antagonist averaging about 0.00008 to 0.0024 mg/kg/week IgE antagonist , or about 0.00025 to 0.0024 mg/kg/week IgE antagonist, or about 0.0008 to 0.0024 mg/kg/week IgE antagonist, for every IU/ml baseline free IgE in the patient's serum. In other embodiments, the maintenance dose averages about 0.00075 to 0.0024 mg/kg/week, or about 0.00125 to 0.0024 mg/kg/week, or about 0.00175 to 0.0024 mg/kg/week, of IgE antagonist for every IU/ml baseline free IgE in the patient's serum. Preferably, the maintenance dose is at least about three fold lower, or at least about six fold lower, or at least about 12 fold lower, or at least about 25 fold lower, or at least about 50 fold lower, than the loading dose in units of mg/kg/week for every IU/ml baseline free IgE in the patient's serum. The patient's reactivity to intranasal allergen challenge can be determined as described above.

The invention also provides methods for reducing reactivity to intranasal allergen challenge in a patient comprising adminstering a loading dose of an IgE antagonist for a period of at least about 14 days, or about 28 to 56 days, or about 42 to 56 days, followed by a maintenance dose of the IgE antagonist that maintains the patient's serum free IgE concentration at a level no greater than about 600 ng/ml, or no greater than about 300 ng/ml, or no greater than about 150 ng/ml, or no greater than about 75 ng/ml, or no greater than about 50 ng/ml, wherein the loading dose exceeds the maintenance dose by at least three fold, or by at least about six fold, or by at least about 12 fold, or by at least about 25 fold, or by at least about 50 fold, in units of mg/kg/week IgE antagonist for every IU/ml baseline free IgE in the patient's serum. The patient's reactivity to intranasal allergen challenge can be determined as described above. The patient's serum free IgE response to the maintenance regimen can be determined by assaying serum free IgE according to the methods for baseline serum free IgE assay described above.

In these embodiments, a typical loading dose used is at least about 0.003 mg/kg/week IgE antagonist, or at least about 0.007 mg/kg/week IgE antagonist, or at least about 0.021 mg/kg/week IgE antagonist, or about 0.003 to 0.030 mg/kg/week IgE antagonist, or about 0.007 to 0.021 mg/kg/week IgE antagonist, for every IU/ml baseline free IgE in the patient's serum. However, the invention also encompasses the use of other loading doses that are sufficient to strip basophils of IgE receptor such that maintenance dose levels which provide the serum free IgE levels recited in the methods are sufficient to substantially maintain IgE receptor suppression and avoid reoccurrence of symptoms. Suitable maintenance dosing for practice of these methods include maintenance doses of about 0.00008 to 0.0024 mg/kg/week IgE antagonist, or about 0.00025 to 0.0024 mg/kg/week IgE antagonist, or about 0.0008 to 0.0024 mg/kg/week IgE antagonist, for every IU/ml baseline free IgE in the patient's serum. In other embodiments, the maintenance dose averages about 0.00075 to 0.0024 mg/kg/week, or about 0.00125 to 0.0024 mg/kg/week, or about 0.00175 to 0.0024 mg/kg/week, of IgE antagonist for every IU/ml baseline free IgE in the patient's serum.

The invention further provides methods for reducing reactivity to skin prick allergen challenge in a patient comprising administering a loading dose of an IgE antagonist for a period of at least about 14 days,

or at least about 28 days, or at least about 42 days, sufficient to reduce the patient's average serum free IgE level to a level no greater than 50 ng/ml, or no greater than 30 ng/ml, or no greater than 16 ng/ml, or no greater than 10 ng/ml, or no greater than 6 ng/ml, at the end of the loading period, followed by a maintenance dose of the IgE antagonist averaging about 0.00008 to 0.0024 mg/kg/week, or about 0.00025 to 0.0024 mg/kg/week, or about 0.0008 to 0.0024 mg/kg/week, of IgE antagonist for every IU/ml IgE in the patient's serum. In other embodiments, the maintenance dose averages about 0.00075 to 0.0024 mg/kg/week, or about 0.00125 to 0.0024 mg/kg/week, or about 0.00175 to 0.0024 mg/kg/week, of IgE antagonist for every IU/ml baseline free IgE in the patient's serum. Preferably, the maintenance dose is at least about three fold lower, or at least about six fold lower, or at least about 12 fold lower, or at least about 25 fold lower, or at least about 50 fold lower, than the loading dose in units of mg/kg/week for every IU/ml baseline free IgE in the patient's serum. In these embodiments, the patient's serum free IgE response to the loading regimen can be determined by assaying the patient's serum free IgE according to the methods for baseline serum free IgE assay described above. The patient's reactivity to skin prick allergen challenge can be determined according to the methods of Bousquet and Michel, "IN VIVO METHODS FOR STUDY OF ALLERGY: Skin tests, techniques and interpretation" *Allergy Principles & Practice,* Middleton et al., eds, pps. 573-594 (1992).

In the practice of the foregoing methods for reducing reactivity to skin prick allergen challenge, the patient's serum IgE level can be reduced to the prescribed level at the end of the loading period by administering for the prescribed loading period a loading dose of at least about 0.003 mg/kg/week of the IgE antagonist, or at least about 0.007 mg/kg/week of the IgE antagonist, or at least about 0.021 mg/kg/week of the IgE antagonist, or about 0.003 to 0.030 mg/kg/week of the IgE antagonist, or about 0.007 to 0.021 mg/kg/week of the IgE antagonist, for every IU/ml baseline free IgE in the patient's serum. However, the invention also encompasses the use of other loading doses that are sufficient to strip basophils of IgE receptor such that maintenance dose levels provided in the methods are sufficient to substantially maintain IgE receptor suppression and avoid reoccurrence of symptoms.

The invention additionally provides methods for reducing reactivity to skin prick allergen challenge in a patient comprising administering a loading dose of an IgE antagonist averaging at least about 0.003 mg/kg/week IgE antagonist, or at least about 0.007 mg/kg/week IgE antagonist, or at least about 0.021 mg/kg/week IgE antagonist, or about 0.003 to 0.030 mg/kg/week IgE antagonist, or about 0.007 to 0.021 mg/kg/week IgE antagonist, for every IU/ml baseline free IgE in the patient's serum for a period of at least about 14 days, or about 14 days to 56 days, or about 21 days to 56 days, or about 28 days to 56 days, or about 35 days to 56 days, or about 42 days to 56 days, or about 49 days to 56 days, followed by a maintenance dose of the IgE antagonist averaging about 0.00008 to 0.0024 mg/kg/week IgE antagonist , or about 0.00025 to 0.0024 mg/kg/week IgE antagonist, or about 0.0008 to 0.0024 mg/kg/week IgE antagonist, for every IU/ml baseline free IgE in the patient's serum. In other embodiments, the maintenance dose averages about 0.00075 to 0.0024 mg/kg/week, or about 0.00125 to 0.0024 mg/kg/week, or about 0.00175 to 0.0024 mg/kg/week, of IgE antagonist for every IU/ml baseline free IgE in the patient's serum. Preferably, the maintenance dose is at least about three fold lower, or at least about six fold lower, or at least about 12 fold lower, or at least about 25 fold l wer, or at least about 50 fold lower, than the loading dose in units of mg/kg/week for every IU/ml

baseline free IgE in the patient's serum. The patient's reactivity to skin prick allergen challenge can be determined as described above.

The invention also provides methods for reducing reactivity to skin prick allergen challenge in a patient comprising administering a loading dose of an IgE antagonist for a period of at least about 14 days, or about 28 to 56 days, or about 42 to 56 days, followed by a maintenance dose of the IgE antagonist that maintains the patient's serum free IgE concentration at a level no greater than about 600 ng/ml, or no greater than about 300 ng/ml, or no greater than about 150 ng/ml, or no greater than about 75 ng/ml, or no greater than about 50 ng/ml, wherein the loading dose exceeds the maintenance dose by at least about three fold, or by at least about six fold, or by at least about 12 fold, or by at least about 25 fold, or by at least about 50 fold, in units of mg/kg/week IgE antagonist for every IU/ml baseline free IgE in the patient's serum. The patient's reactivity to skin prick allergen challenge can be determined as described above. The patient's serum free IgE response to the maintenance regimen can be determined by assaying serum free IgE according to the methods for baseline serum free IgE assay described above.

In these embodiments, a typical loading dose used is at least about 0.003 mg/kg/week IgE antagonist, or at least about 0.007 mg/kg/week IgE antagonist, or at least about 0.021 mg/kg/week IgE antagonist, or about 0.003 to 0.030 mg/kg/week IgE antagonist, or about 0.007 to 0.021 mg/kg/week IgE antagonist, for every IU/ml baseline free IgE in the patient's serum. However, the invention also encompasses the use of other loading doses that are sufficient to strip basophils of IgE receptor such that maintenance dose levels which provide the serum free IgE levels recited in the methods are sufficient to substantially maintain IgE receptor suppression and avoid reoccurrence of symptoms. Suitable maintenance dosing for practice of these methods include maintenance doses of about 0.00008 to 0.0024 mg/kg/week IgE antagonist, or about 0.00025 to 0.0024 mg/kg/week IgE antagonist, or about 0.0008 to 0.0024 mg/kg/week IgE antagonist, for every IU/ml baseline free IgE in the patient's serum. In other embodiments, the maintenance dose averages about 0.00075 to 0.0024 mg/kg/week, or about 0.00125 to 0.0024 mg/kg/week, or about 0.00175 to 0.0024 mg/kg/week, of IgE antagonist for every IU/ml baseline free IgE in the patient's serum.

The invention further encompasses methods for reducing the average number of FcεRI receptors expressed per basophil in an atopic patient's serum comprising administering to an atopic patient a loading dose of an IgE antagonist over a period of at least about 14 days, or at least about 28 days, or at least about 42 days, sufficient to reduce the average number of FcεRI receptors expressed per basophil in the patient's serum at the end of the loading period to a number that is substantially lower, or at least about 90% lower, or at least about 95% lower, or least about 99% lower, than the baseline average number of FcεRI receptors expressed per basophil in the patient's serum, followed by a maintenance dose of the IgE antagonist sufficient to maintain the average number of FcεRI receptors expressed per basophil in the patient's serum at a number that is substantially lower, or at least about 90% lower, or at least about 95% lower, or at least about 99% lower, than the baseline average number of FcεRI receptors expressed per basophil in the patient's serum, wherein the loading dose exceeds the maintenance dose by at least about three fold, or by at least about six fold, or by at least about 12 fold, or by at least about 25 fold, or at least about 50 fold, in units of mg/kg/week for every IU/ml baseline free IgE in the patient's serum. In these embodiments, the patient's FcεRI expression

levels at baseline and at various points during the l ading and maintenance regimens can be determined as described in Malveaux *et al., supra*, or as described in Example 1 below.

In these embodiments, a typical loading dose used is at least about 0.003 mg/kg/week IgE antagonist, or at least about 0.007 mg/kg/week IgE antagonist, or at least about 0.021 mg/kg/week IgE antagonist, or about 0.003 to 0.030 mg/kg/week IgE antagonist, or about 0.007 to 0.021 mg/kg/week IgE antagonist, for every IU/ml baseline free IgE in the patient's serum. Suitable maintenance dosing for practice of these methods include maintenance doses of about 0.00008 to 0.0024 mg/kg/week IgE antagonist, or about 0.00025 to 0.0024 mg/kg/week IgE antagonist, or about 0.0008 to 0.0024 mg/kg/week IgE antagonist, for every IU/ml baseline free IgE in the patient's serum. In other embodiments, the maintenance dose averages about 0.00075 to 0.0024 mg/kg/week, or about 0.00125 to 0.0024 mg/kg/week, or about 0.00175 to 0.0024 mg/kg/week, of IgE antagonist for every IU/ml baseline free IgE in the patient's serum. However, it will be appreciated that the invention also encompasses the use of other loading doses and/or maintenance doses that are capable of effecting and maintaining the suppression of basophil FcεRI expression prescribed in the present methods.

Typically, IgE antagonists are administered by intravenous, subcutaneous, intramuscular, or intraperitoneal injection, or by other parenteral routes, or by intrabronchial or intranasal inhalation. Other suitable routes of administration are also encompassed within the scope of the invention. It is envisioned that injections (intravenous, subcutaneous or intramuscular) will be the primary route for therapeutic administration of the IgE antagonist of the invention, although delivery through catheter or other surgical tubing can also be used. Alternative routes include suspensions, tablets, capsules and the like for oral administration, commercially available nebulizers for liquid formulations, and inhalation of lyophilized or aerosolized microcapsules, and suppositories for rectal or vaginal administration. Liquid formulations can be utilized after reconstitution from powder formulations. The IgE antagonists can be administered before and/or after the onset of symptoms.

As provided herein, the particular loading dose, loading dose period, maintenance dose, and maintenance dose period selected for an individual patient within the methods of the invention are determined according to good medical practice taking into account the properties of the IgE antagonist employed, e.g. its in vivo plasma half-life, the formulation of the IgE antagonist employed, the disorder to be treated, the condition of the individual patient, the clinical tolerance of the patient involved, and the like, as is well within the skill of the physician. It will be understood that individual patients require different levels of free IgE clearance to produce the desired level of basophil stripping (down-regulation of IgE receptor). Also, individual patients may respond differently to the IgE antagonist used. Thus, individual patients may require different levels of drug in order to achieve the desired effect. Such variations are well within the skill of the treating physician, and are encompassed by the scope of the invention as described and claimed herein. Aside from any variation in absolute dosing levels that may be required among individual patients, the underlying biology remains the same for each patient such that the basophil stripping caused and maintained by the methods of the invention will result in improvement in clinical outcome while using significantly less drug than would be indicated under an ordinary treatment regimen.

Although the invention encompasses the treatment of any allergic disease according to the methods described herein, the preferred indications include allergic asthma and allergic rhinitis. The present methods for therapy of allergic rhinitis, allergic asthma and other allergic diseases can be combined with known therapies for allergy, asthma or other allergic diseases, including treatments with anti-histamines, theophylline, salbutamol, beclomethasone dipropionate, sodium cromoglycate, corticosteroids, anti-inflammatory agents, and the like.

Further details of the invention can be found in the following examples, which further define the scope of the invention. All references cited throughout the specification, and the references cited therein, are hereby expressly incorporated by reference in their entirety.

EXAMPLES

EXAMPLE 1

Materials and Methods

Buffer: PIPES (Piperazine-N,N-bis-2-ethanesulfonic acid) (Sigma Chemical Co., St. Louis, MO) stock buffer, 25 mM PIPES containing 110 mM NaCl, 5 mM KCl, and 40 mM NaOH, adjusted to pH 7.3 and stored at 10 times the above concentration. PAG: PIPES (1X) containing 0.003% human serum albumin (HSA) (Miles Laboratories Inc., Elkhart, IN) and 0.1% glucose. PAGCM: PAG with 1.0 mM $CaCl_2$ and 1.0 mM $MgCl_2$. PAG-EDTA: PAG with 1.0 mM (ethylenediaminetetraaceticacid (EDTA). Acetate elution buffer contained 0.05 M sodium acetate, 0.085 M NaCl, 10 mM EDTA and 0.03% HSA at pH 7.3.

Reagents: Polyclonal goat anti-human IgE was prepared as described by Adkinson, in "Measurement of total serum immunoglobulin E and allergen-specific immunoglobulin E antibody" (2nd ed), Washington, D.C.: *American Society of Microbiology* (1980), p. 800; the antibody used for these studies represented the IgG fraction of goat serum prepared by DE-52 chromatography. Dust mite antigen (5000 protein nitrogen units (PNU)/ml, containing 0.4% phenol), D. farinae (Miles, Inc.) was obtained as the skin test reagent used to screen the donors used in the study. This antigen was therefore dialyzed against 3 changes of saline and finally 1X PIPES buffer using SpectroPor 10,000 MWCO cutoff tubing (Spectrum Medical Industries, Los Angeles, CA). The resulting antigen was tested on three types of donors, a non-releaser (no IgE-mediated histamine release with anti-IgE antibody), a highly sensitive non-atopic donor and an atopic donor without sensitivity to dust mite antigens. The dialyzed antigen did not induce release in these three types of donors while it was an effective stimulus for basophils from dust mite allergic donors. The optimal concentration on the dose response curve was determined to be approximately 10 PNU/ml for dust mite allergic donors. FMLP (formyl-methionyl-leucyl-phenylalanine) was obtained from Sigma. Benzylpenicilloyl(BPO)-specifidgE was partially purified from the serum of a penicillin allergic patient by affinity methods described by MacGlashan, *J. Immunol.*, **130**: 2337-2342 (1983). It is a combination of both IgG and IgE specific for penicillin and >95% of the IgE is specific for the penicillin hapten. BPO(11)-HSA was prepared as described by MacGlashan, *J. Immunol.*, **127**: 2410-2414 (1981). Purified IgE - PS myeloma was obtained as described in Ishizaka, *Immunochemistry*, 7: 687-702 (1970).

Subjects: Fifteen subjects were recruited through public advertisement for participation in the open-labeled, Phase I trial of intravenous recombinant human monoclonal antibody E25 (rhuMAb-E25). They all

complained of perennial nasal symptoms and fulfilled the following entry criteria: age range of 18-65, negative serum pregnancy test (βHCG) at entry and usage of an acceptable method of contraception for females, weight within the 70 to 130% range of the ideal value for their height using the 1983 Metropolitan Height and Weight Table, positive (>5 mm wheal) epicutaneous skin test to either Dermatophagoides farinae or Dermatophagoidespteronnysinus, serum total IgE ranging between 85 and 550 IU/ml and a stable therapeutic regimen. Eleven subjects were also affected by asthma. Subjects were excluded if they had ever experienced anaphylaxis, if their rhinitis included marked seasonal exacerbations, if they had to be hospitalized or visit the emergency department for asthma treatment within 6 weeks prior to the initiation of the study or if they had required mechanical ventilation for status asthmaticus in the year prior to the initiation of the study. In addition, individuals were excluded whose baseline forced expired volume in one second (FEV_1) was <50% of the predicted value, or who had been on allergy immunotherapy in the year prior to the study, or who had a history of a medical illness that raised reasonable suspicion on possible interference with the safety outcomes of the study. Pregnancy or lactation were also exclusion criteria.

Nine patients received a loading dose of rhuMAb-E25, followed by maintenance dosing equivalent to 7.5 μg/kg/week per IU/mL baseline IgE. Three patients received maintenance doses of 15 μg/kg/week per IU/mL baseline IgE. Two controls were also enrolled, however, these donors were only monitored for receptor density changes, i.e., they did not receive any form of placebo therapy.

Cell preparation: Two types of basophil preparation were employed. For the receptor measurements by acid elution, venous blood was drawn by venipuncture and the cells were separated on a single-step Percoll gradient as described by MacGlashan, *J. Immunol.*, **124**: 2519-2521 (1980). Briefly, blood was centrifuged over Percoll (specific gravity=1,080xg), then the mononuclear cell layer was collected and washed with EDTA saline, PAG-EDTA, and twice with PAG, and then used as described below. For the flow cytometric studies, the blood was diluted with Percoll to a final density of 1.065 g/ml and layered over Percoll at a density of 1.079 g/ml as described by Warner, *J. Immunol. Methods*, **105**: 107-110 (1987). After centrifugation at 450xg for 15 minutes the interface between the 1.065 Percoll/plasma upper layer and the 1.079 lower Percoll layer was harvested and washed as above.

General Protocol: For receptor measurements by acid elution, washed mononuclear cells were divided into 3 portions, 2/9 for histamine release, 2/9 for endogenous IgE density measurements, and 5/9 for sensitization (see below) with subsequent IgE density measurements. The cells destined for histamine release were challenged as soon as the cells were finished being prepared in order to minimize the effects of handling on intrinsic functional responses of the basophils. The cells destined for determination of endogenous IgE densities were resuspended in 5 ml of PAG buffer and incubated at 37°C for 30 minutes, after which they were centrifuged and resuspended in 1 ml saline. Further processing for IgE densities is described below. The cells destined for sensitization were prepared as described below.

Cell challenge: Dose response curves for polyclonal anti-IgE antibody were examined to obtain the optimal or maximum release value. The response to two doses of D. farinae previously shown to be optimal and suboptimal for release (10 and 0.5 PNU/ml, respectively) was included. To determine the response of the basophils to a n n-IgE mediated stimulus and to determine if this response remained constant through ut

therapy, cells were challenged with 1 μM FMLP. Cells were resuspended in PAGCM, then challenged with stimulus for 45 minutes at 37°C at a final volume of 1.0 ml. All reactions, performed in duplicate, were stopped by centrifugation after 45 minutes and the supernatant was removed for histamine analysis. In each experiment, perchloric acid at a 1.6% final concentration was added to duplicate tubes to determine total histamine content. Histamine was assayed by the automated fluorometric technique of Siraganian and Brodsky, *J. Clin. Immunol.* 57: 525-540 (1976) and the percentage of histamine release was calculated from the ratio of sample to total histamine after spontaneous release was subtracted from both.

Sensitization: For cells used to determine both the total and unoccupied receptor densities, the cells were first sensitized with penicillin-(BPO) specific IgE. The cells were incubated with IgE antibody at 5 μg/ml for 20 minutes at 37°C in RPMI-1640 media (Life Technologies, Gaithersberg, MD) containing 1 mM EDTA and 10 μg/ml heparin (250 μl final volume). Previous studies demonstrated that sensitization with this concentration of BPO-specific IgE, for this length of time, effectively saturates unoccupied receptors and therefore allowed a measurement of total receptors (by measuring the total IgE from saturated cells) as well as measurement of the unoccupied receptor density (MacGlashan, *J. Immunol.*, 130: 2330-2336 (1983)). After washing once, the cells were layered over 1 ml of chelated FCS (5 mM EDTA in heat inactivated fetal calf serum) and centrifuged for 5 minutes to rapidly separate the cells from their diluted sensitization buffer. The cells were further washed twice in PAG and then resuspended in 5 ml of PAG buffer and incubated for 60 minutes at 37°C. This incubation step or the one used for unsensitized cells (see above) were incorporated to facilitate the elution of IgE from low affinity receptor binding, whether it be to FcεRII or FcγRII, as determined in previous studies. After centrifugation, the pellets were resuspended in 1 ml of saline.

Acetate elution: Prior to a final centrifugation of the cells suspended in saline (saline is used to eliminate the buffering capacity of the PIPES, reducing its influence on the subsequent acetate elution), duplicate samples were taken for cell counts. For the cells that were sensitized and after centrifugation, a sample of the saline supernatant was kept for analysis of IgE to ensure that there was no carry over from the sensitization step. None of these samples contained measurable BPO-specific IgE. The pellets were resuspended in ice-cold acetate buffer, pH 3.7, and incubated in an ice bath for 10 minutes. For the cells destined for a determination of endogenous IgE, the elution volume was 0.35 ml and for the cells used for total and unoccupied receptors the final volume of elution was 0.7 ml. After a brief centrifugation (15,000 Xg), the supernatant was removed, and neutralized with 1 N NaOH. These samples were frozen immediately for later measurement of total and specific IgE by radioimmunosorbent test (RIST) or radioallersorbent test (RAST) as described by Adkinson, in "Measurement of total serum immunoglobulin E and allergen-specific immunoglobulin E antibody", 2nd ed., Washington, D.C.: *American Society of Microbiology* (1980), p. 800. Acid elution was done in duplicate. In addition to these samples, each day of testing also included a sample of IgE treated with the acetate buffer (or not treated as a control) and these samples were also frozen with the cell elution samples. These later samples were used to determine if acid treatment and storage affected the measurement of IgE, however, no effect was observed. Basophil counts (determined by alcian blue staining according to the method of Gilbert, *Blood*, 46: 279-286 (1975), made before elution, were also done in duplicate. This method of acetate "stripping" determines the amount of IgE bound to mononuclear cells but

previous studies have shown that it is the basophil that is the primary, if not sole, contributor to "stripped" IgE antibody.

IgE measurement: The eluted IgE was measured in either a total IgE RIST (endogenous IgE or total receptor measurement) or BPO-RAST (unoccupied receptors, see below). Cells counts (alcian blue p sitive cells) obtained prior to elution allowed a calculation of the receptor density to be made (the amount of IgE measured by RIST or RAST divided by cell count with the result expressed as IgE molecules per basophil). This general procedure and the acetate elution technique are described in detail by MacGlashan, *J. Immunol.*, **127**: 2410-2414 (1981); Conroy, *J. Immunol.*, **118**: 1317-1321 (1977); MacGlashan, *J. Immunol.*, **136**: 2231-2239 (1986).

Flow Cytometry: At various timepoints throughout the study, human basophils were isolated from 10 ml of venous blood by Percoll density gradient centrifugation as described by Warner, *J. Immunol. Methods*, **105**: 107-110 (1987). Basophil purity ranged between 1 and 9% (based on light microscopic counts after alcian blue staining). A flow cytometric technique incorporating light scatter characteristics was used to quantitate cell surface IgE and FcεRIα chain expression on basophils as described by Bochner, *J. Immunol. Methods*, **125**: 265-271 (1989). Cell surface IgE was detected using fluorescein isothiocyanate (FITC)-conjugated polyclonal goat IgG anti-human IgE (Kirkegaard and Perry, Gaithersberg, MD); FITC-conjugated polyclonal normal goat IgG (Kirkegaard and Perry) was used as a control. To fully saturate FcεRI, separate aliquots of cells were passively sensitized with PS IgE myeloma (10 µg/ml, 37°C, 15 minutes in PAG/EDTA/heparin buffer, see above) prior to immunofluorescent labeling. Cell surface expression of FcεRIα chain was detected using a mouse IgG1 anti-human FcεRIα chain monoclonal antibody (29C6) obtained as described in Riske, *J. Biol. Chem.*, **266**: 11245-11251 (1991) and was compared to labeling with an irrelevant mouse IgG1 (Coulter, Hialeah, FL). The 29C6 antibody has been shown to recognize an epitope that is unaffected by FcεRI occupancy (Riske, *J. Biol. Chem.*, **266**: 11245-11251 (1991)). Aliquots of cells were labeled in phosphate buffered saline containing 0.2% bovine serum albumin (BSA) with 4 mg/ml human IgG to minimize nonspecific binding to FcγR as described by Bochner, *J. Immunol. Methods*, **125**: 265-271 (1989). Binding of monoclonals was detected using saturating concentrations of phycoerythrin-conjugated polyclonal goat anti-mouse IgG (Tago, Burlingame, CA). An EPICS Profile flow cytometer was used to analyze fluorescent signals after excitation at 488 nM. The use of "bitmap" gates to select for a population of cells which are predominantly basophils is possible because basophils have reasonably distinctive forward and side scatter characteristics. Since the cells were also enriched using a double Percoll gradient, these bitmaps can select a population of cells that is generally greater than 80% basophils, with the primary contaminants being lymphocytes. Monocytes were also gated for study by their distinctive forward/side scatter characteristics. Data is expressed as the mean fluorescence in labeled cells minus the mean fluorescence of IgG1 controls. Of note, incubation of fresh basophils from naive donors with up to 200 µg/ml of E25, *in vitro*, failed to alter labeling of basophils with FITC-anti-IgE or 29C6 antibodies, suggesting that E25 does not interfere with immune fluorescent labeling. For some samples, 100 µl of whole blood was treated with perchoric acid and histamine content analyzed as above. These samples were used to determine if histamine content was measurable even if IgE fluorescence became undetectable.

Statistics: A non-parametric Mann Whitney U rank order statistic was generally examined for each of the data sets although in some instances simple paired t-tests were also applied.

Results

Twelve atopic subjects with baseline serum IgE concentrations between 85 IU/mL and 550 IU/mL were administered rhuMAb-E25 intravenously. Baseline characteristics of the study participants are noted in Table 1. All subjects received regular administration of rhuMAb-E25 during the course of this study.

Table 1: Baseline characteristics of study subjects

Age range (mean ± SD)	29.8 ± 6.3
Sex male female	 7 5
Weight (kg) (mean ± SD)	77.2 ± 16.3
Baseline IgE (IU/mL) (mean ± SD)	192 ± 79
Maintenance dose of rhuMAb-E25 mg/wk (mean ± SD)	57.1 ± 20.2

The data in Figure 1 show serum titers of free IgE pre-dose and two hours post-dose on the first day of therapy and on 7, 14, 28, and 42 days after the start of therapy for the 12 atopic patients. Free IgE titers dropped immediately after the first dose and were essentially constant thereafter, averaging 1% of the pre-therapy titers.

The protocol for receptor density measurements was carried out just prior to the start of therapy and 3 months after the start of therapy. Figure 2 shows the results for the three parameters measured. Fig. 2A plots the basophil surface IgE densities for each patient. As noted in the Methods section above, it was previously established that little, if any, of the IgE measured by this technique comes from cells other than basophils. The pre-therapy average was approximately 250,000 IgE molecules per basophil. At the three month time point, the amount of IgE "stripped" from the cells and measured in the total IgE RIST was not detectable for most of the treated patients. Since basophils were counted, a maximum value for the IgE density could be assigned. The 0.512 ng/ml standard on the standard curve for the total IgE RIST represented the least detectable concentration (≥2 standard deviations from blanks). Therefore this value was used in the numerator of the expression to calculate IgE density (amount of IgE/basophil count). In Fig. 2, the "stars" represent data calculated in this manner, and it applies to all but one treated donor at the three month time point for the data in Fig. 2A. While a true average can not be calculated since most of the three month data represents only calculated maximums for each patient, the average of the data calculated in this manner was approximately 2200 IgE molecules per basophil, a greater than 99% decrease ($p=.0001$ by Mann-Whitney U test).

Fig. 2B shows a similar set of data for sensitized cells, i.e., a measurement of IgE density after saturating the unoccupied receptors and therefore a measurement of total receptor density. It can be seen that all samples had measurable IgE after "stripping," indicating a detectable number of total receptors. The average receptor number before sensitization was again approximately 250,000 IgE receptors per cell. This was reduced to approximately 8600 IgE receptors per basophil at the three month measurement. Thus, after therapy there were greater than three fold more unoccupied than occupied receptors, but like the plasma IgE levels and the endogenous basophil surface IgE densities, total receptor density had markedly decreased, by approximately 97% (Mann-Whitney U, p=.0001).

Fig. 2C shows data for the unoccupied receptors before and during treatment, i.e., the "stripped" IgE from sensitized cells measured by the BPO-RAST. Since the IgE used for sensitization was nearly 100% specific for penicillin, this measurement determines the unoccupied receptor density. Prior to therapy this density averaged approximately 2700 IgE receptors per basophil while after therapy, the average increased to 7100/basophil. This increase was statistically significant (Mann-Whitney U, p=.0001). The difference between total receptors (Fig. 2B) and unoccupied receptors (Fig. 2C) for the three month time point, using the average values, suggests a value of approximately 1500 endogenous IgE molecules which is just below the maximum estimate obtained in Fig. 2A. However, it should also be pointed out that this acetate "stripping" method of measuring receptors has an average error of approximately 25%, so that the average total receptor density at three months of 8600, if calculated from an individual experiment, would have a one standard deviation error of ±2200. Therefore, the difference of 1500 should be interpreted cautiously. At the pre-therapy and three month time points, basophil numbers were not significantly different, with an average ratio 1.06±.11 counts at 3 months counts pre-therapy.

At three times during therapy, the leukocytes were also analyzed for IgE by flow cytometry. As noted in Figure 3, cells were studied just prior to therapy, 5 weeks, and 10 weeks after the start of therapy. Flow cytometry, as described in the **Methods** section above, provided an important control to rule out the possibility that the absolute receptor measurements were also assessing high affinity binding of IgE on cells other than basophils, i.e., on monocytes. The enriched cells were examined with or without prior sensitization with IgE myeloma to determine the endogenous expression of IgE or total receptor expression. As can be seen in Figure 3A, by 5 weeks, and persisting at 10 weeks, the cells of most donors expressed very little endogenous IgE; most positively stained flow cytometric distributions became indistinguishable from control staining distributions (Mann-Whitney U, p=.0001). These points are plotted as a zero in the figure. Interestingly, the one subject with persistently detectable levels of IgE (Figure 3A) was the same subject in which a definitely detectable level of IgE was found by RIST (Figure 2A). Total receptor expression (Figure 3B, after passive sensitization) also decreased rapidly but was detectable at five weeks for many patients while it became generally undetectable by 10 weeks. It should be pointed out that in previous calibrations of the sensitivity of the flow cytometer for detecting basophil IgE, the minimum detectable expression was found to be 8000-10000 IgE receptors per basophil. Therefore these undetectable levels of expression are compatible with the data shown in Figure 2. Indeed, the sporadic net positive results (among patients) after

sensitizati n are also compatible with the unoccupied receptor data in Figure 2 since the average total receptor density borders on the threshold of detection for the flow cytometer.

For the last three patients enrolled in the study, flow cytometric measurements were made several times during the first two weeks of therapy in order to more carefully examine the kinetics of IgE and FcεRI down-regulation. The dosing regimen was slightly different for these three patients and the timing of the doses is indicated in Figure 4. Figure 4 demonstrates that the decrease in both endogenous IgE density and total receptors occurred with a half life of approximately three days for all three patients. In these experiments, the total receptor expression was also determined more directly using an anti-FcεRIα subunit antibody, 29C6, which binds to both occupied and unoccupied receptors (Fig. 4B). Reduction in FcεRI paralleled the total receptor data obtained by first sensitizing the cells with IgE myeloma (Fig. 4A). Both endogenous IgE and FcεRI decreased at similar rates. Flow cytometric distributions remained unimodal throughout the two week time course.

Figure 5 illustrates the functional consequences of these changes on basophils as measured by their ability to secrete histamine in response to several physiologic stimuli. The basophils were assessed before and during therapy by challenge with 1) dust mite antigen, D. farinae, at two doses, optimal and suboptimal, 2) a full dose response to polyclonal goat anti-IgE antibody and 3) a single concentration of FMLP. Since FMLP acts through a distinct receptor that has a markedly different signal transduction pathway from IgE-mediated release, the response to this stimulus was not expected to change during therapy and, indeed, as can be seen in the right side histogram in Fig. 5, it did not. In contrast, the response to dust mite antigen (the response to an optimal concentration is shown) decreased by approximately 90% (median ratio of post/pre was 0.09, p=.0002 by Mann-Whitney U). In fact, in half the patients there was essentially no response. The response to the 20 fold lower suboptimal concentration of dust mite antigen was proportionally lower or zero. The response of the two controls used in these studies was essentially unchanged (ratio of post/pre was 0.83±.25 (range, n=2)). The response to anti-IgE antibody was also decreased in the treated patients but not to same extent as antigen, with the peak response decreasing approximately 40% (p=.046 by Mann Whitney U) and no meaningful change in the character of the dose response curve. There was significant variability among donor basophil responses, with nearly no change in the response to anti-IgE antibody in some donors, while in several cases the response to anti-IgE was nearly ablated. The response of the basophils that had been sensitized with anti-BPO IgE (for the receptor studies) to both an optimal concentration of anti-IgE (0.2 μg/ml) and to an optimal concentration of BPO(11)-HSA was determined for seven of the patients at the three month time point. On average, the histamine release to BPO-HSA was 70±7%, essentially the same as the pre-therapy response to the natural antigenic sensitivity, D. farinae (78±7%). The response to anti-IgE antibody also increased to 52±8%, which is also the same as the pre-therapy response to an optimal concentration of anti-IgE antibody (53±7%). These studies indicate that the number of unoccupied receptors remaining on the treated patients' basophils were sufficient to drive a normal response to both antigen and anti-IgE when sensitized (see Discussion below). It was not possible to properly correlate the responses of the cells to remaining (endogenous) IgE densities because, as noted above, the three month data generally only represented the maximum possible IgE densities. However, there were two anecdotal observations.

First, the only patient wh se basophils had a measurable endogenous IgE density (the nly circle point in Figure 1A at "90" days) was also the only patient whose response to dust mite antigen was only marginally reduced. Second, the tw patients whose estimated "maximum" endogenous IgE densities were the lowest (1400-1500/basophil) also showed strong responses to anti-IgE antibody but poor responses to dust mite antigen.

Discussion

These studies demonstrate a remarkable down-regulation of FcεRI on basophils during a period of time when the free circulating titers of IgE reached a small fraction of the patients' pre-therapy levels. Both measures of FcεRI expression on basophils, absolute quantitation by acetate stripping and flow cytometry, resulted in the same conclusion. Although the number of controls used was small, many previous studies have shown that IgE and FcεRI densities are relatively stable over long periods of time, varying at most 2-3 fold but usually varying no more than the error of this measurement technique (MacGlashan, *J. All. Clin. Immunol.*, **91**: 605-615 (1993).

The kinetics of the decrease in IgE expression, under conditions where the free IgE concentration was less than 1 ng/ml, indicate that the dissociation constant for FcεRI was on an scale similar to that found for the interaction of rat IgE antibody and rat FcεRI ($T_{1/2}$ approximately 50-70 hrs) (Kulczycki, *J. Exp. Med.*, **140**: 1676-1695 (1974); Metzger, *Immunochemistry*, **13**: 417-423 (1976)). The data does not support the possibility that this loss represents the replacement of an original pool of basophils with new basophils that did not experience up-regulation of FcεRI because of the low plasma IgE concentration. The observation that the flow cytometric distributions for samples taken within the first several days of therapy were unimodal suggests that there were not two populations of basophils, but rather that the existing population was uniformly losing IgE.

These observations have several implications with respect to the observed changes in receptor density as they relate to the changes in function. Previous studies have noted that basophils have many "spare" receptors. Basophils from a typical patient will give a half maximal response to antigenic stimulation with a cell surface density of 2000 antigen-specific IgE molecules (MacGlashan, *J. Immunol.*, **91**: 605-615 (1993)). As noted in this study, the starting density of IgE was approximately 250,000 molecules and since the ratio of antigen-specific IgE to total IgE can be as high as 50% (in ragweed allergic patients for example), there will clearly be many "spare" IgE molecules. In order to reduce the presence of antigen-specific IgE on the basophil surface to levels significantly below this EC_{50} threshold, in the absence of a change in receptor density, total IgE titer would have to decrease at least 100 fold. Factoring in the down-regulation of the receptor, however, suggests that the decrease in IgE titer need be much less profound. As an illustration, given the following conditions: 1) a patient naturally expressing 250,000 FcεRI molecules, 2) occupied with IgE which was 25% specific for ragweed antigens, 3) a typical basophil sensitivity of 2000 molecules for a half maximal response, 4) using a log-linear extrapolation to a minimum of 8300 FcεRI molecules as the IgE titer decreased, 5) a starting total IgE of 100 ng/ml and 6) an equilibrium constant for IgE:FcεRI of 10^{10} to decrease the antigen specific IgE to the EC_{50}, or 2000 molecules per cell, it would require a decrease in plasma IgE concentration to 0.63 ng/ml (1/160) without a change in receptors and a decrease to 6.7 ng/ml

(1/15) with the change in receptors, i.e. an approximately 99% decrease vs an approximately 90% decrease. Therefore, the responsiveness of FcεRI expression to IgE antibody concentration accentuates the ability of the therapy to down-regulate the overall responsiveness of the basophil.

EXAMPLE 2

A placebo-controlled, double-blind study is performed to determine the safety, pharmacokinetics, pharmacodynamics and efficacy of long-term dosing of anti-IgE recombinant humanized monoclonal antibody E25 (rhuMAb-E25) (described as humanized variant 12 of monoclonal antibody MAE11 in Presta et al., **and also described as variant 8b in Table 9 on page 68 of Ser. No. 08/405,617**) following short term high-dose loading in patients with allergic rhinitis or allergic asthma. In particular, the objectives are to evaluate the safety of rhuMAb-E25 administered by high-dose loading in patients with perennial allergic rhinitis or asthma, and to determine if a significant reduction in dose of rhuMAb-E25 can maintain suppression of basophil FcεRI expression and clinical outcomes.

The study can include approximately 150 to 200 patients with history of allergic rhinitis or allergic asthma, and who are positive skin prick test-positive to dust mite allergens (greater than 5 mm wheal and erythema). Exclusion criteria are FEV1 less than or equal to 50% of the predicted FEV1 at screening, use of topical nasal cromolyn or corticosteroids for 14 days before, or during the course of the study, pregnant women or women of child-bearing potential not on an accepted form of contraception, or chronic disease (e.g. diabetes mellitus, hypertension, chronic obstructive pulmonary disease (COPD), coronary artery disease (CAD).

Serum concentrations of total serum IgE and free serum IgE can be measured at baseline and at various time points during the study. Total IgE can be measured using a commercially available kit (IMx Total IgE, Abbot Laboratories, Abbott Park, IL). Serum free IgE can be measured using solid phase ELISA. High binding flat bottom polystyrene plates (Costar, Cambridge, MA) are coated overnight at 2-8°C with 100 ng of human IgE receptor α-chain IgG chimera (FcεRI-IgG) (obtained as described in Haak-Frendscho et al., J. Immunol., 151: 351 (1993)) in 100 µl of pH 9.6 carbonate buffer. The plates are then washed with 0.05% Tween 20 in phosphate-buffered saline (PBS) and incubated with 200 µl of assay diluent (0.5% bovine serum albumin (BSA) [Intergen Co., Purchase, NY]/0.05% Tween 20/0.01% thimerosal in PBS) for 1 to two hours. The plates are sealed and frozen until use. All subsequent assay steps are performed at room temperature. For the ELISA, plates are thawed and washed, and 50 µl of sample or standard are added in duplicate to 50 µl of assay diluent in the wells and incubated for one hour. FcεRI-IgG captures only free IgE (IgE that is not complexed with E25). The plates are washed, and 100 ng of biotinylated monoclonal anti-human IgE in 100 µl of assay diluent is added to the wells and incubated for one hour. The plates are washed and 100 µl of avidin-horseradish peroxidase (HRP) (Vector Laboratories, Burlingame, CA) diluted 1/2000 in assay diluent is added to the wells and incubated for 30 minutes. The plates are washed and then developed with 0.4 mg/ml o-phenylenediamine and 4 mM H_2O_2 in PBS. The color reaction is stopped with 4.5 N H_2SO_4. The absorbance at 492 nm is measured with an SLT EAR 340AT (Research Triangle Park, NC). The concentration of free IgE is calculated for the standard curve, using a 4-parameter logistic fit for the standards.

In the asthmatic arm of the study, the primary efficacy variable is the change in FEV1 measured within one hour of allergen provocative bronchial challenge (EAR) between day -1 and after final administration of the study drug. The baseline is defined as the observed difference in the percent change from prechallenge levels in FEV1 response between an allergen diluent challenge and an allergen challenge. The follow-up is defined as the difference in the percent change from prechallenge levels in FEV1 response between an allergen diluent challenge and an allergen challenge after final administration of the study drug. In each case, two variables are derived: maximal observed decrease and area under the curve (AUC) as approximated by the trapezoidal rule. Treatment efficacy is based on the between treatment comparison of the baseline and follow-up of AUC and maximal increase. Between group differences for the change between day -1 and post-final drug administration are assessed by the Wilcoxon Rank sum test.

LAR is measured in a similar fashion as the primary efficacy variable of change in the FEV1 (AUC and maximal decrease).

The bronchoprovocation tests can be conducted as follows. The initial dosing of allergen for inhalation is four doubling doses below that calculated from the prediction formula: $y = 0.69x + 0.11$, where $y = \log_{10}$ allergen PD_{20}FEV1 (the dose of allergen that causes a 20% fall in FEV1) and $x = \log_{10}$ methcholine PD_{10} (the dose of methocholine that cases a 10% fall in FEV1) multiplied by skin allergen sensitivity (skin sensitivity to allergen is defined as the smallest allergen dilution that gives a wheal 2 mm in diameter). When the dose causes a fall in FEV1 of 20% or more, no further allergen is delivered. When the dose causes a fall in FEV1 of less than 10%, then the challenge is advanced to the next doubling step, etc. The FEV1 is measured at 20, 30, 45, 60, 90 and 120 minutes and at hourly intervals up to seven hours after inhalation.

Dosing of allergen for the follow-up bronchial challenge commences at an allergen concentration of four doubling doses more dilute than the dose which caused a 20% fall in FEV1 during the first challenge. The dosing then proceeds in two-fold more concentrated steps until the FEV1 falls by 20% or until allergen is delivered at a concentration one doubling dose higher than that delivered on day -1.

In the rhinitis arm of the study, the primary efficacy variable is the change in clinical symptom score for nasal response to allergen provocative intranasal challenge from day -1 to various times during treatment and after final administration of the study drug. The nasal challenge tests can be conducted as follows. An atomized allergen solution, beginning with the lowest concentration (highest dilution), is applied to each nasal mucosa only, with no portion of the dose delivered beyond the posterior nasal mucosa. Based on the patient's tolerance, atomized allergen solution is reapplied using the next most concentrated (10 fold base increase from the lowest concentration) until the onset of symptoms listed in Table 2 below are exhibited. Usually, the first symptom is marked by nasal congestion or discomfort.

Table 2: Clinical Symptom Score for Nasal Response

Symptom	Response	Points
Sneezes	0	0
	1-3	1
	>3	2
Rhinorrhea	0	0
	mild	1
	abundant	2
Congestion	0	0
	mild	1
	nasal block	2
Itching	0	0
	itchy eyes and/or throat	1
	watery and itchy eyes, and itchy palate and throat	2

Upon determination of a positive clinical challenge score of greater than or equal to four points, the challenge procedure is complete. A positive clinical challenge score is defined as greater than four points (maximum possible score is eight). Treatment efficacy is assessed by comparison of pre-treatment, during-treatment and post-treatment nasal symptom scores.

In both arms of the study (asthmatic and rhinitic), treatment efficacy is assessed by allergen-titrated skin prick tests conducted at various times before, during and after treatment with the study drug.

In both arms of the study, basophils are harvested from the patients at various times before, during and after treatment and assayed for FcεRI expression as described in Example 1 above.

In both arms of the study, patients are randomized to receive one of the following two loading regimens or placebo as set forth in Table 3 below. Loading doses are expressed in units of mg/kg/week E25 administered for every IU/ml baseline free IgE in the patient's serum. Patients can receive the loading dose in one or more administrations by intravenous or subcutaneous injection during the loading period.

Table 3: rhuMAb-E25 Loading Regimens

Loading Dose	Duration of Load
High: 0.021	4-6 weeks
Low: 0.007	4-6 weeks

After completing the loading regimen, patients in each loading group receive one of the maintenance regimens (or placebo) shown in Table 4 below. Loading doses are expressed in units of mg/kg/week E25 administered for every IU/ml baseline free IgE in the patient's serum. Patients can receive

the maintenance dose by intravenous or subcutaneous injection given at any convenient combination of dose amount and time interval, e.g., administering four times the weekly dose once every four weeks.

Table 4: rhuMAb-E25 Maintenance Regimens

High Loading Group	Low Loading Group
0.007	0.0024
0.0024	0.0008
0.0008	0.00025
0.00025	Placebo
Placebo	

The above-described therapeutic treatment of atopic patients with E25 is expected to reduce reactivity to bronchoprovocation, nasal challenge, and skin test with allergen, maintain suppression of basophil FcεRI expression, and improve clinical outcomes.

WE CLAIM:

1. A method of treating an *allergic disease* selected from the group consisting of allergic *rhinitis* and allergic *asthma* in a patient comprising administering;

a) a **loading dose** of an IgE antagonist for a period of at least about 14 days sufficient to reduce the patient's average serum free IgE level to a level no greater than about 50 ng/ml at the end of the loading period; followed by,

b) a **maintenance dose** of the IgE antagonist averaging about 8×10^{-5} to 2.4×10^{-3} mg/kg/week IgE antagonist for every IU/ml baseline free IgE in the patient's serum;

wherein the **maintenance dose** is *at least about three fold lower* than the **loading dose** in units of mg/kg/week IgE antagonist for every IU/ml baseline free IgE in the patient's serum.

2. The method of claim 1 wherein the loading dose is sufficient to reduce the patient's serum IgE level to an average level no greater than about 30 ng/ml at the end of the loading period.

3. The method of claim 2 wherein the loading dose is sufficient to reduce the patient's serum IgE level to an average level no greater than about 16 ng/ml.

4. The method of claim 3 wherein the loading dose is sufficient to reduce the patient's serum IgE level to an average level no greater than about 10 ng/ml.

5. The method of claim 4 wherein the loading dose is sufficient to reduce the patient's serum IgE level to an average level no greater than about 6 ng/ml.

6. A method of treating an *allergic disease* selected from the group consisting of allergic *rhinitis* and allergic *asthma* in a patient comprising administering;

a) a **loading dose** of an IgE antagonist averaging at least about 3×10^{-3} mg/kg/week IgE antagonist for every IU/ml/ baseline free IgE in the patient's serum for a period of at least about 14 days; followed by,

b) a **maintenance dose** of the IgE antagonist averaging about 8×10^{-5} to 2.4×10^{-3} mg/kg/week IgE antagonist for every IU/ml baseline free IgE in the patient's serum;

wherein the **maintenance dose** is *less than about one third* of the **loading dose**.

7. The method of claims 1 & 6 wherein the maintenance dose of the IgE antagonist averages about 0.00025 to 0.0024 mg/kg/week IgE antagonist for every IU/ml baseline free IgE in the patient's serum.

8. The method of claims 1 & 6 wherein the maintenance dose of the IgE antagonist averages averages about 0.0008 to 0.0024 mg/kg/week IgE antagonist for every IU/ml baseline free IgE in the patient's serum.

9. The method of claim 6 wherein the loading dose of the IgE antagonist averages at least about 0.007 mg/kg/week IgE antagonist for every IU/ml baseline free IgE in the patient's serum.

10. The method of claim 9 wherein the loading dose of the IgE antagonist averages at least about 0.021 mg/kg/week.

11. A method of treating an *allergic disease* selected from the group consisting of allergic *rhinitis* and allergic *asthma* in a patient; comprising administering,

(a) a **loading dose** of an IgE antagonist for a period of at least about 14 days, followed by;

(b) a **maintenance dose** of the IgE antagonist that maintains the patient's serum free IgE concentration at a level no greater than about 600 mg/ml;

wherein the **loading dose** exceeds the maintenance does by at least about three fold in units of mg/kg/week IgE antagonist for every IU/ml baseline free IgE in the patient's serum.

12. The method of claim 11 wherein the loading dose exceeds the maintenance dose by at least about six fold in units of mg/kg/week IgE antagonist for every IU/ml baseline free IgE in the patient's serum.

13. The method of claim 12 wherein the loading dose exceeds the maintenance dose by at least twelve fold.

14. The method of claim 13 wherein the loading dose exceeds the maintenance dose by at least twenty-five fold.

15. The method of claim 14 wherein the loading dose exceeds the maintenance dose by at least fifty fold.

16. The method of claim 11 wherein the maintenance dose of the IgE antagonist maintains the patient's serum free IgE concentration at a level no greater than about 300 ng/ml.

17. The method of claim 16 wherein the patient's free IgE concentration is maintained at a level no greater than about 150 ng/ml.

18. The method of claim 17 wherein the patient's free IgE concentration is maintained at a level no greater than about 75 ng/ ml.

19. The method of claim 18 wherein the patient's free IgE concentration is maintained at a level no greater than about 50 ng/ml.

20. The method of claims 1, 6 and 11 wherein the loading dose is administered for a period of 14-56 days.

21. The method of claim 20 wherein the period is 21-56 days.

22. The method of claim 21 wherein the peirod is 28-56 days.

23. The method of claim 22 wherein the period is 35-56 days.

24. The method of claim 23 wherein the period is 42-56 days.

25. The method of claim 24 wherein the period is 49-56 days.

26. The method of claims 1, 6 and 11 wherein the IgE antagonist is an anti-IgE antibody.

27. The method of claim 26 wherein the antibody is chimeric.

28. The method of claim 27 wherein the antibody is humanized.

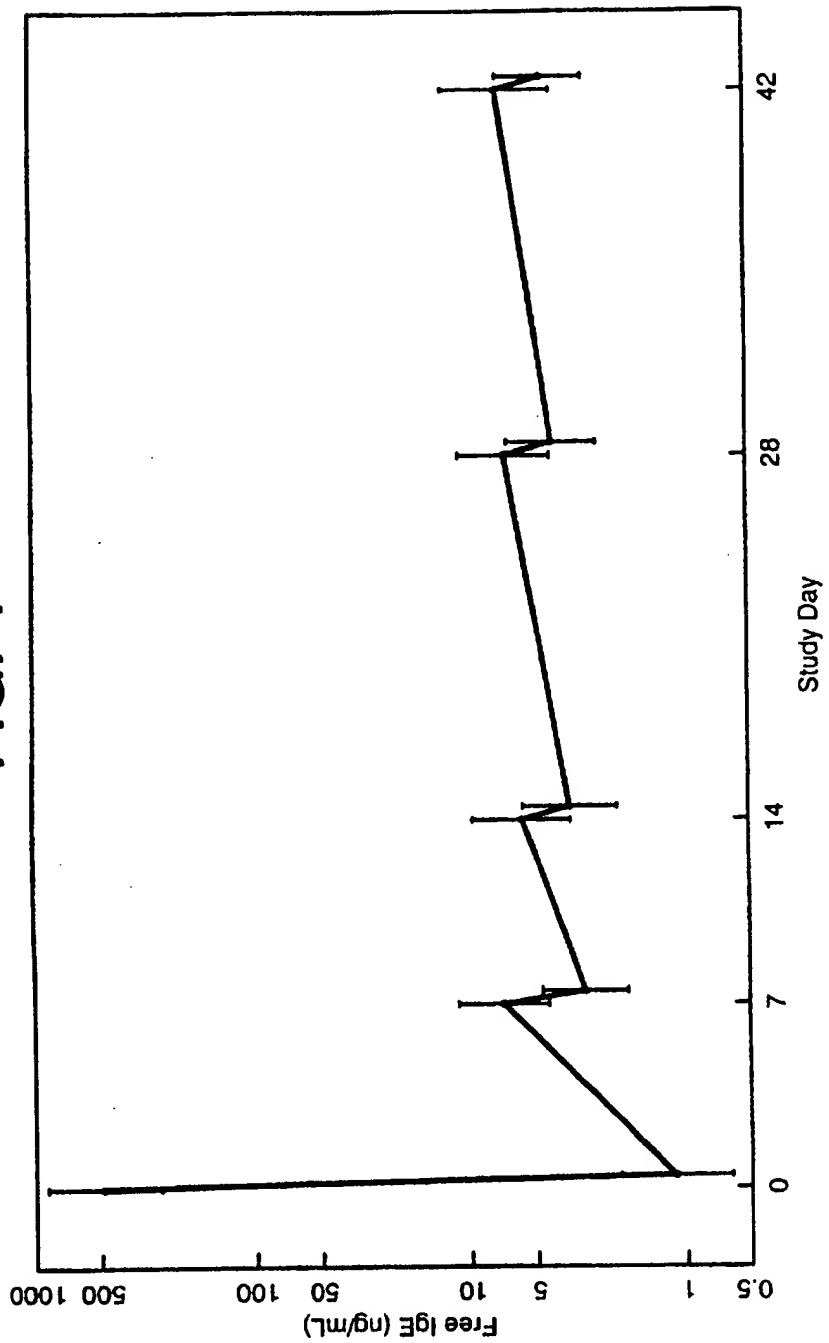
29. The method of claim 26 wherein the antibody is a human antibody.

30. The method of claim 28 wherein the antibody is rhuMAb-E25.

31. The method of claim 26 wherein the antibody binds to soluble IgE and blocks the binding of IgE to the IgE receptor on basophils.

32. The method of claim 26 wherein the antibody binds to membrane bound IgE.

FIG. 1



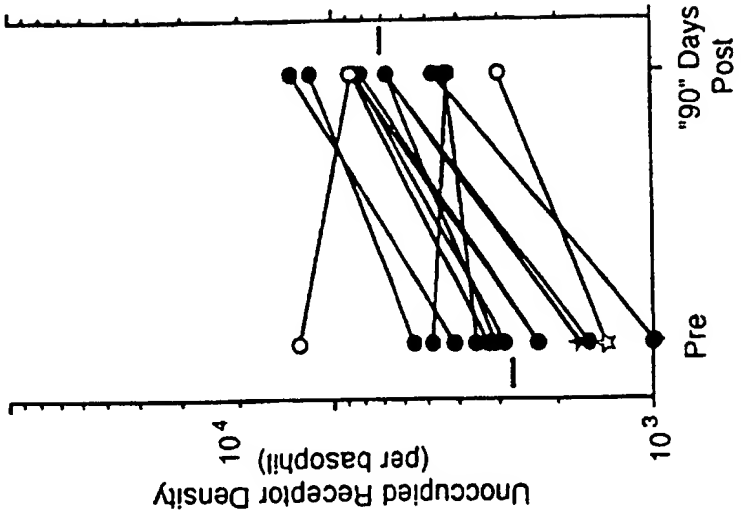


FIG. 2C

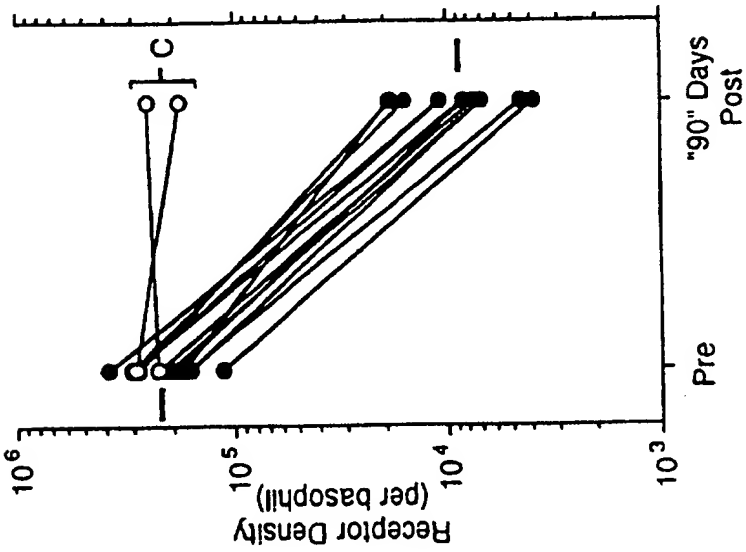


FIG. 2B

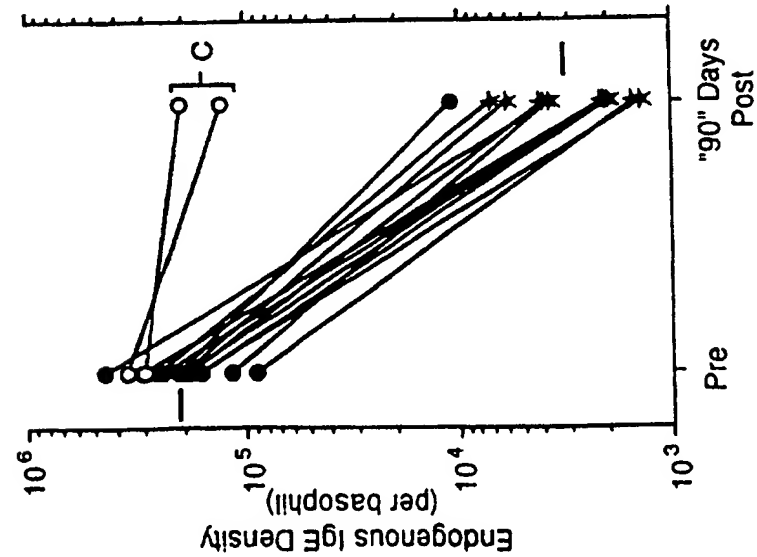


FIG. 2A

FIG. 3A

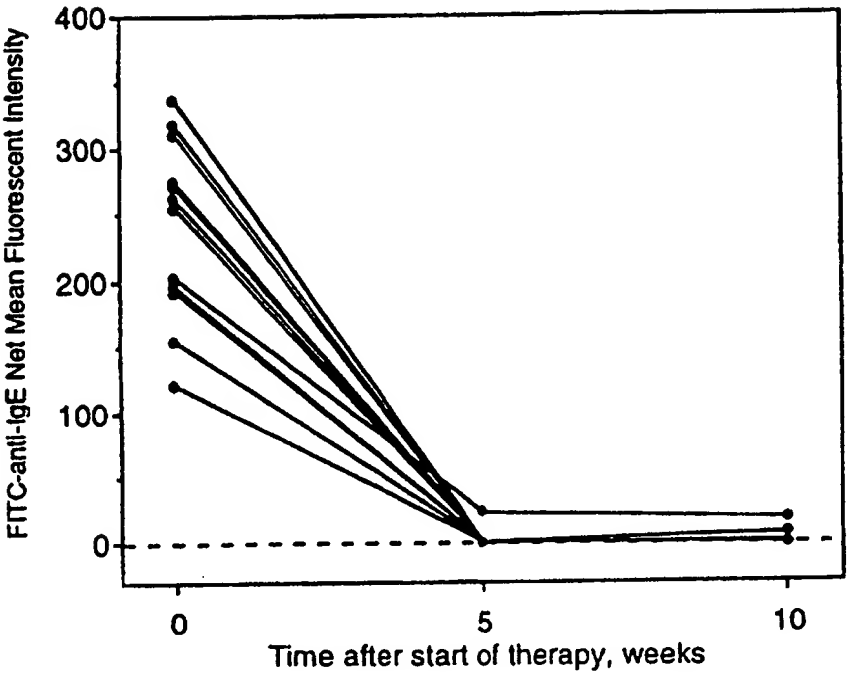

400
300
200
100
0
FITC-anti-IgE Net Mean Fluorescent Intensity
0
5
10
Time after start of therapy, weeks

FIG. 3B

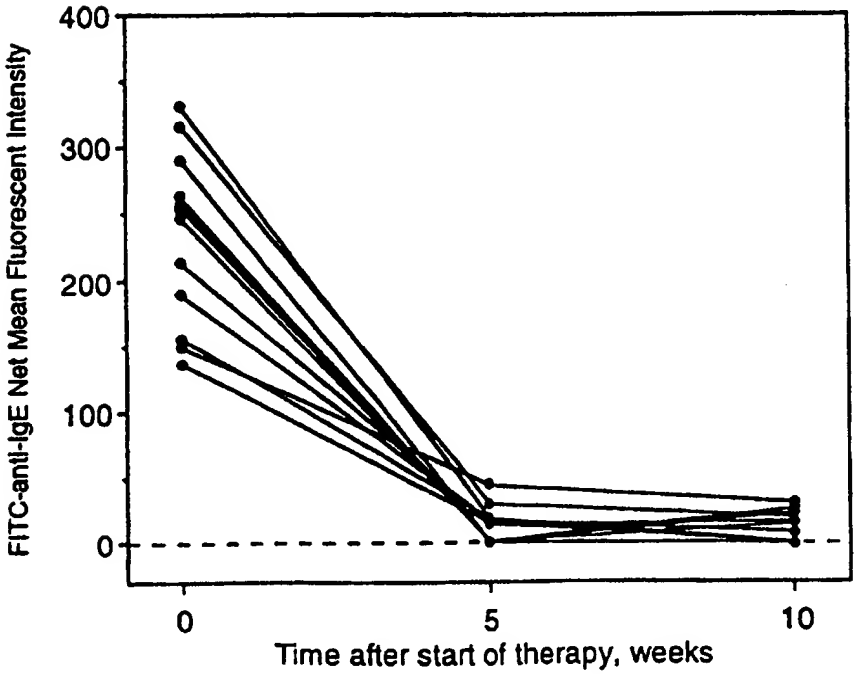

400
300
200
100
0
FITC-anti-IgE Net Mean Fluorescent Intensity
0
5
10
Time after start of therapy, weeks

FIG. 4A

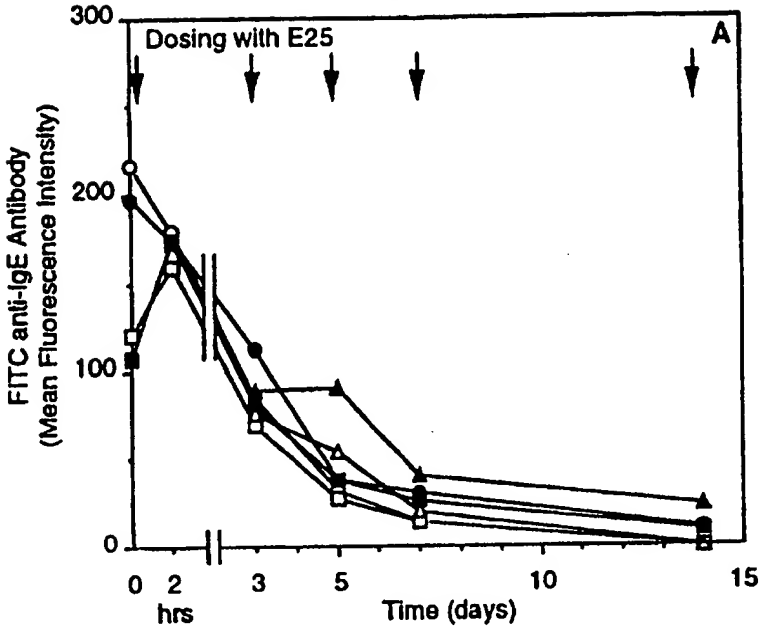
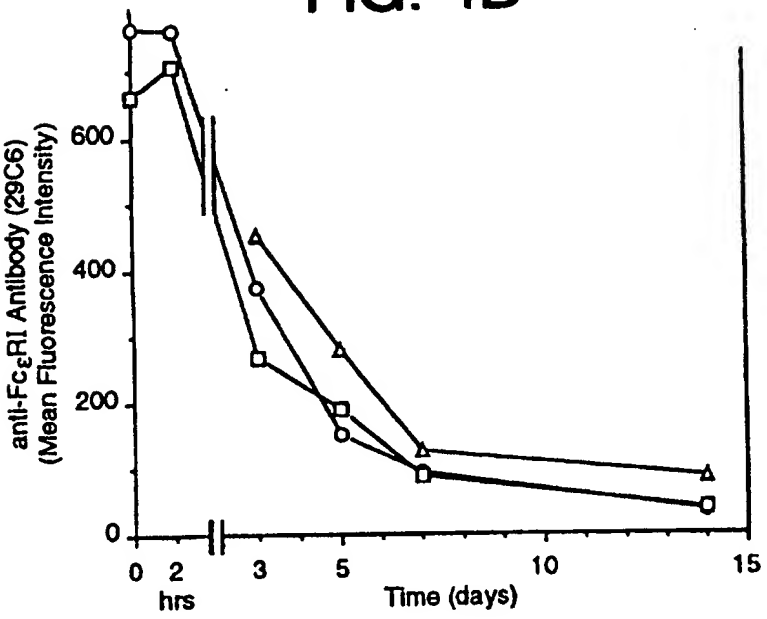

Dosing with E25
A
FITC anti-IgE Antibody
(Mean Fluorescence Intensity)
300
200
100
0
0 2
hrs
3
5
10
15
Time (days)

FIG. 4B


anti-FcεRI Antibody (29C6)
(Mean Fluorescence Intensity)
600
400
200
0
0 2
hrs
3
5
10
15
Time (days)

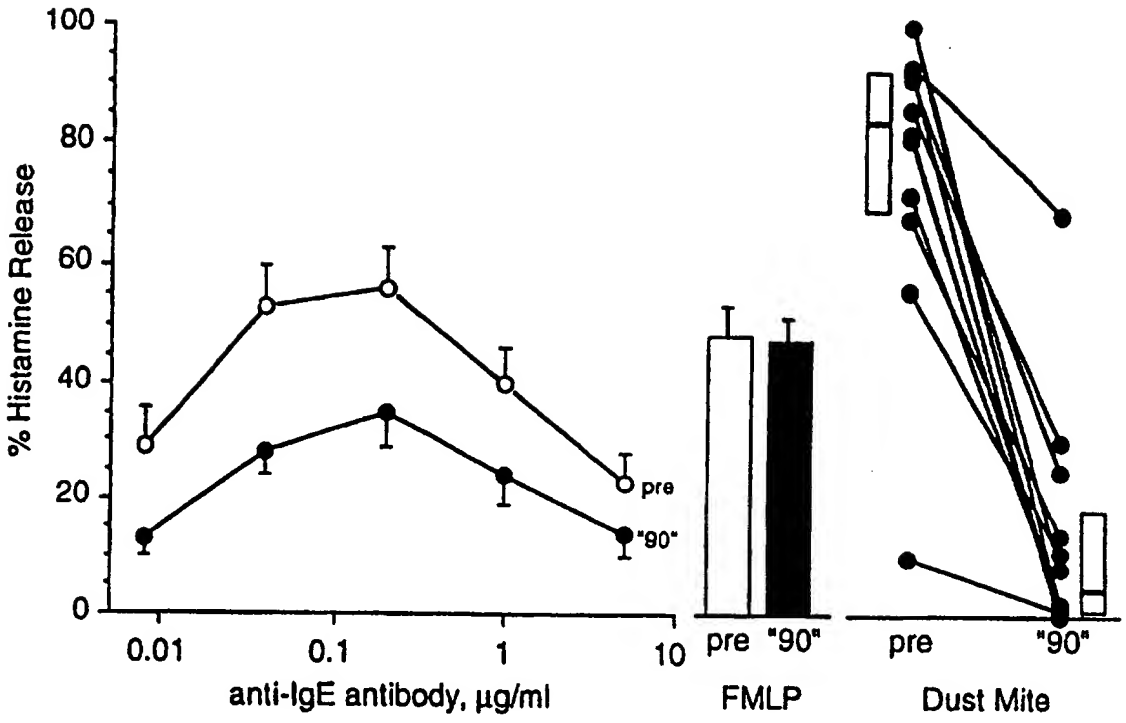

100
80
60
40
20
0
% Histamine Release
pre
"90"
0.01
0.1
1
10
anti-IgE antibody, µg/ml
pre
"90"
FMLP
pre
"90"
Dust Mite

FIG. 5

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/03443

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/395 C07K16/42

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07K C12N C12P G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 550 020 A (SNOW BRAND MILK PROD CO LTD) 7 July 1993 see page 5, line 3 - line 18 see page 9, line 51 - page 10, last line ---	1-29,31, 32
Y	US 4 940 715 A (KURASHINA YOSHIKAZU ET AL) 10 July 1990 see claim 7 ---	1-29,31, 32
A	THERAPEUTIC IMMUNOLOGY, FEB 1995, 2 (1) P41-52, ENGLAND, XP002035433 JACQUEMIN MG ET AL: "Specific down-regulation of anti-allergen IgE and IgG antibodies in humans associated with injections of allergen-specific antibody complexes." see the whole document --- -/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

2

Date of the actual completion of the international search	Date of mailing of the international search report
17 July 1997	04.08.1997

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Halle, F

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/03443

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 449 760 A (CHANG TSE-WEN) 12 September 1995	

2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/03443

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-32
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 1-32 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

In ational Application No
PCT/US 97/03443

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0550020 A	07-07-93	JP 5199895 A	10-08-93
		CA 2086131 A	25-06-93
		US 5625039 A	29-04-97
		ZA 9210006 A	29-06-93
US 4940715 A	10-07-90	JP 1290676 A	22-11-89
		JP 8005884 B	24-01-96
		JP 1290677 A	22-11-89
		JP 8005883 B	24-01-96
		EP 0343832 A	29-11-89
US 5449760 A	12-09-95	US 5422258 A	06-06-95
		AT 121299 T	15-05-95
		AU 3031089 A	01-08-89
		DE 3853636 D	24-05-95
		EP 0407392 A	16-01-91
		EP 0617127 A	28-09-94
		HK 31096 A	01-03-96
		US 5543144 A	06-08-96
		WO 8906138 A	13-07-89
		US 5614611 A	25-03-97
		US 5420251 A	30-05-95
		US 5428133 A	27-06-95
		US 5342924 A	30-08-94
		US 5514776 A	07-05-96
		US 5260416 A	09-11-93
		US 5274075 A	28-12-93
		US 5252467 A	12-10-93
		US 5231026 A	27-07-93

What Is Claimed Is:

1. A purified oligopeptide that interacts with the Fcε receptor, wherein the oligopeptide comprises the amino acid sequence:

P S P F D L F I R K S.

2. The purified oligopeptide according to claim 1 wherein the oligopeptide is fewer than 50 amino acids in length.

3. The purified oligopeptide according to claim 2 wherein the oligopeptide is 13-27 amino acids in length.

4. The purified oligopeptide according to claim 1, wherein said oligopeptide has been constrained to form a loop structure.

5. A purified oligopeptide comprising an amino acid sequence selected from the group consisting of the sequences set forth in Seq. I.D. Nos. 1-5.

6. A pharmaceutical preparation comprising a purified oligopeptide according to claims 1, 2, 3, 4 or 5.

7. An isolated DNA molecule encoding an oligopeptide according to claims 1, 2, 3, 4 or 5.

8. A biological system that expresses the DNA molecule of claim 7.

9. A biological system according to claim 8, wherein said biological system is a prokaryotic or a eukaryotic cell.

10. A method of preparing an oligopeptide competitor of IgE, comprising:

(a) culturing a prokaryotic or eukaryotic cell according to claim 9, and

(b) purifying said oligopeptide.

11. A competitive binding assay for hIgE wherein the competitor is an oligopeptide according to claims 1, 2, 3, 4 or 5.

12. An antibody raised against a purified oligopeptide according to claims 1, 2, 3, 4 or 5.

13. A pharmaceutical preparation comprising an antibody according to claim 12.

14. A diagnostic method for determining IgE levels in a sample of fluids comprising the steps of:

(a) contacting said sample with an antibody raised against an oligopeptide competitor to IgE; and

(b) precipitating IgE with said antibody.

15. The method of claim 14 wherein the antibody is an antibody raised against a purified oligopeptide according to claims 1, 2, 3, 4, or 5.

16. A diagnostic method of determining available Fcε receptors on the membrane of cells, comprising the steps:

(a) providing a sample of said cells;

(b) providing a labeled oligopeptide competitor to IgE; and

(c) determining the amount of label bound by said cells.

17. A kit for a diagnostic method for determining IgE levels, comprising an antibody of claim 12 and a container for said antibody.

18. A diagnostic kit for determining available Fcε receptors comprising a purified oligopeptide according to claims 1, 2, 3, 4 or 5, and a container for said polypeptide.

19. A method of targeting a cell which expresses FcεR1 receptor, comprising delivering into an organism an oligopeptide according to claim 1.

20. A protein that interacts with the FcεR1 receptor, wherein the protein consists essentially of the amino acid sequence P S P F D L F I R K S.

21. A protein that competes with IgE for the FcεR1 receptor, wherein the protein consists essentially of the amino acid sequence selected from the group consisting of the sequences set forth in Seq. I.D. Nos. 1-5.

22. A protein that competes with IgE for the FcεR1 receptor, wherein the protein consists essentially of:

(i) an amino acid sequence P S P F D L F I R K S; and

(ii) constraining residues that flank the amino acid sequence of (i), wherein the constraining residues include cysteine and are within 16 residues N-terminal and 4 residues C-terminal of the amino acid sequence of (i).

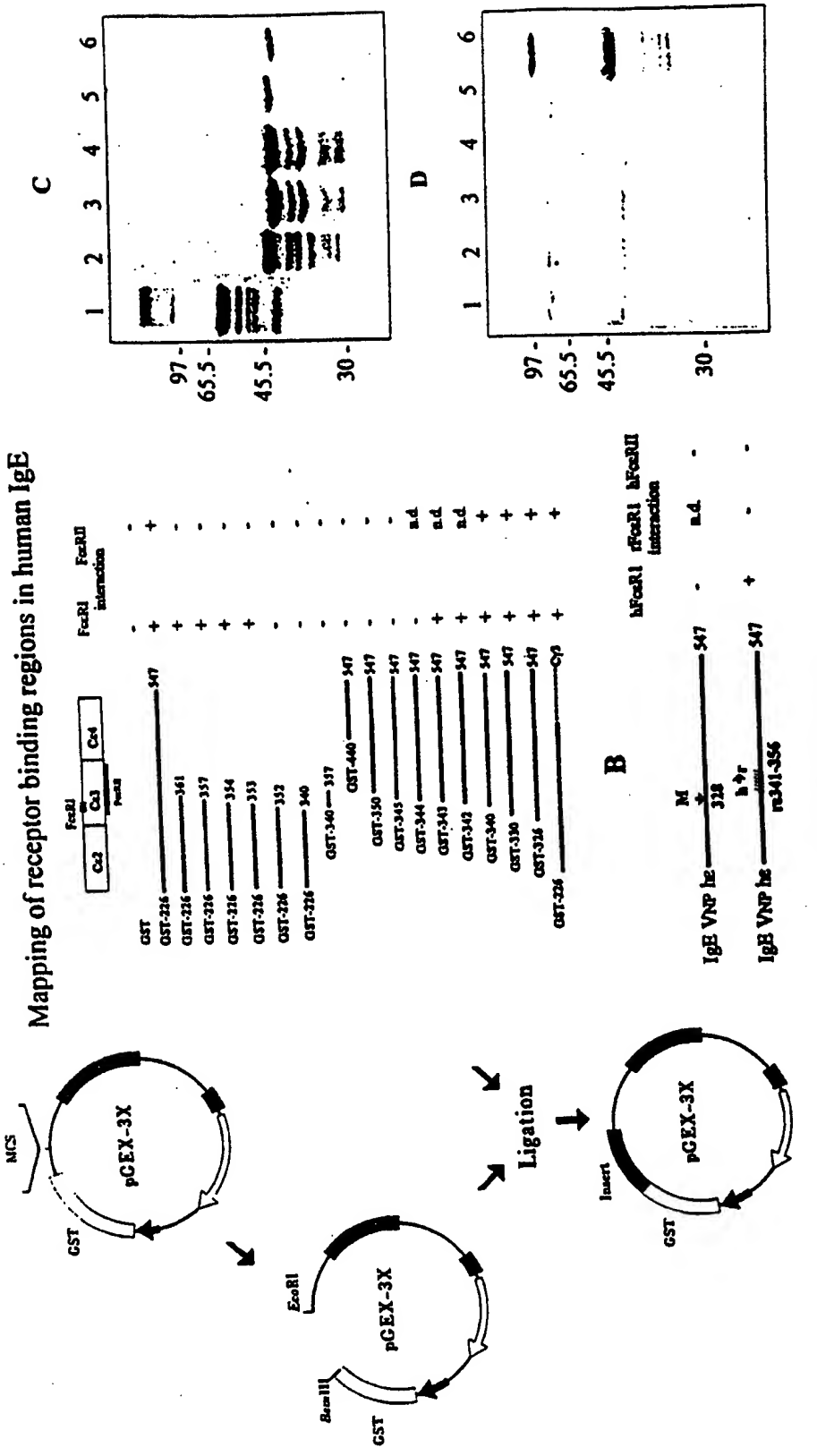

A
Mapping of receptor binding regions in human IgE
Cε2
Cε3
Cε4
FceRI
FceRII
GST
GST-226
GST-336
GST-340
GST-440
GST-350
GST-345
GST-344
GST-343
GST-342
GST-340
GST-330
GST-326
GST-326
B
hFceRI
rFceRI
hFceRII interaction
M
IgE VNP hε
318
h+r
IgE VNP hε
n341-356
n.d.
C
D
97-
65.5-
45.5-
30-
1 2 3 4 5 6
MCS
pGEX-3X
GST
EcoRI
BamHI
Ligation
Insert

FIGURE 1

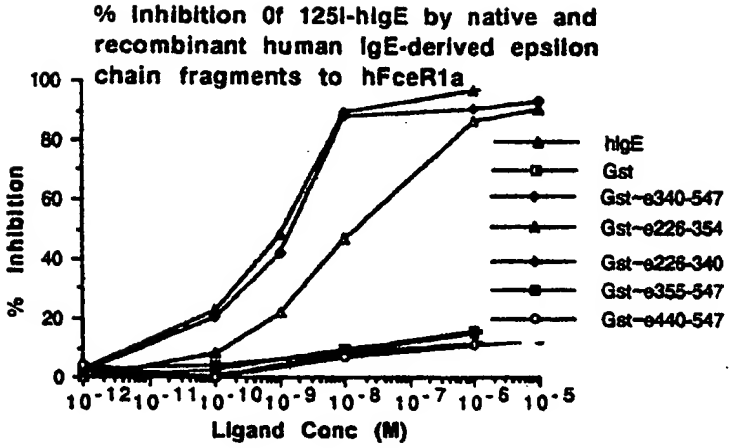

% Inhibition Of 125I-hIgE by native and recombinant human IgE-derived epsilon chain fragments to hFceR1a
% Inhibition
100
80
60
40
20
0
10-12 10-11 10-10 10-9 10-8 10-7 10-6 10-5
Ligand Conc (M)
hIgE
Gst
Gst-e340-547
Gst-e226-354
Gst-e226-340
Gst-e355-547
Gst-e440-547

FIGURE 2

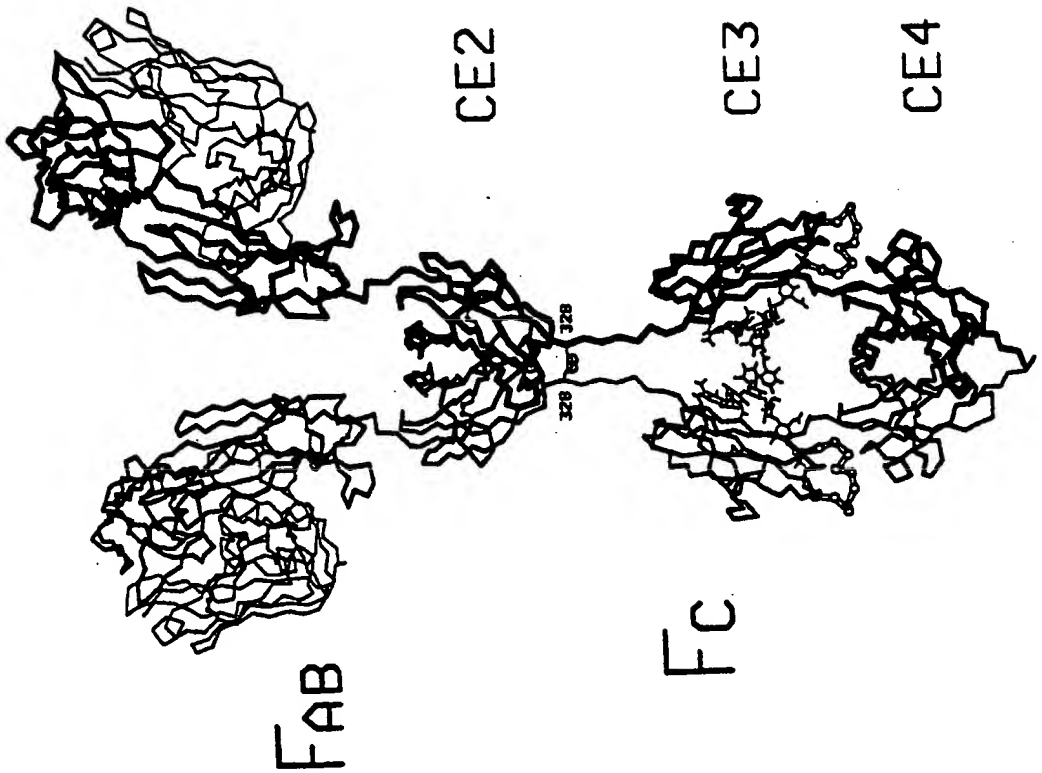


FIGURE 3

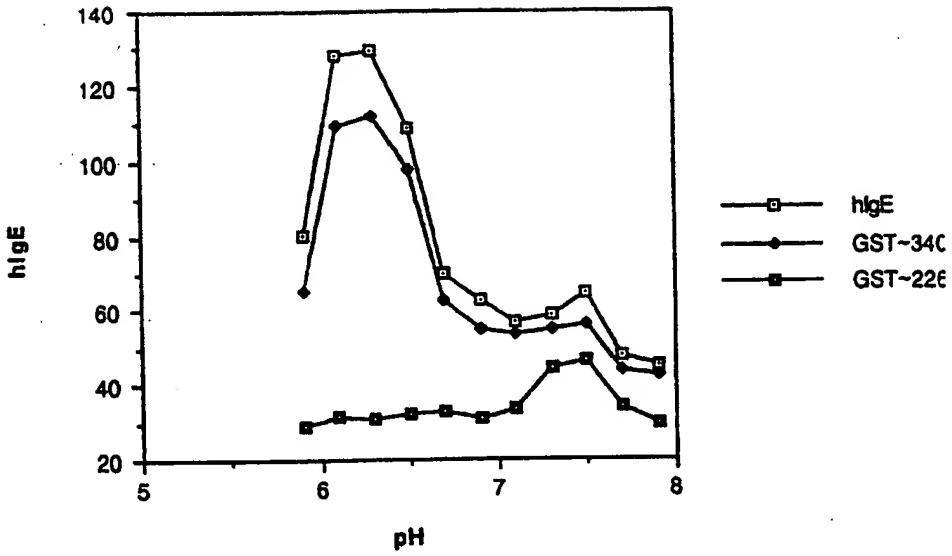


FIGURE 4